

nature

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Being circumspect about plutonium

PLUTONIUM has emerged into the public gaze again in the past year or so not only because of disquieting reports of lax security at places where it is stored in the United States but also because of fears that the radiation safety standards for the substance, in particular its radioactive isotope plutonium-239, do not properly take account of its true toxicity. The former problem could affect many people very quickly—for example if a terrorist group were to steal sufficient plutonium to cobble up a crude bomb—but it admits of a simple solution through improvements in security. The toxicity question is, however, a thorny one, which has implications, in the first instance, for those who work with plutonium, although the matter is by no means irrelevant to the public at large.

Unfortunately for the clarification of the issue, some of the statistics that seem to point to an even higher toxicity than previously thought are not agreed upon—notably those pertaining to the nuclear fuel reprocessing plant of British Nuclear Fuels Limited at Windscale on the north-west coast of England, where five employees, ex-employees and pensioners of the company (which was hived off from the United Kingdom Atomic Energy Authority three years ago) have died of leukaemia, according to company records, in the 24 years the plant has been operating. On the basis of an average 'risk population' of about 3,000 during that period, the company says that the expected natural incidence of leukaemia would be at least four, an indistinguishably different number statistically. Those who take issue with British Nuclear Fuels say that 3,000 is an overestimate, perhaps by a factor of three or more, of those actually at risk and, furthermore, that radiation workers who leave that kind of work altogether are lost from the system of medical monitoring and checks that operates in the nuclear industry (a situation which in itself strongly suggests the need for some sort of national register of people who have ever been radiation workers, perhaps like the Transuranium Registry in the USA).

The statistics mentioned are at odds with each other to the extent that it has been argued that workers associated with plutonium are an order of magnitude more likely to contract leukaemia than people engaged in other occupations. Clearly, the only reasonable basis for new plutonium safety standards is a solid body of research and a careful analysis of the assumptions made in arriving at the present standards.

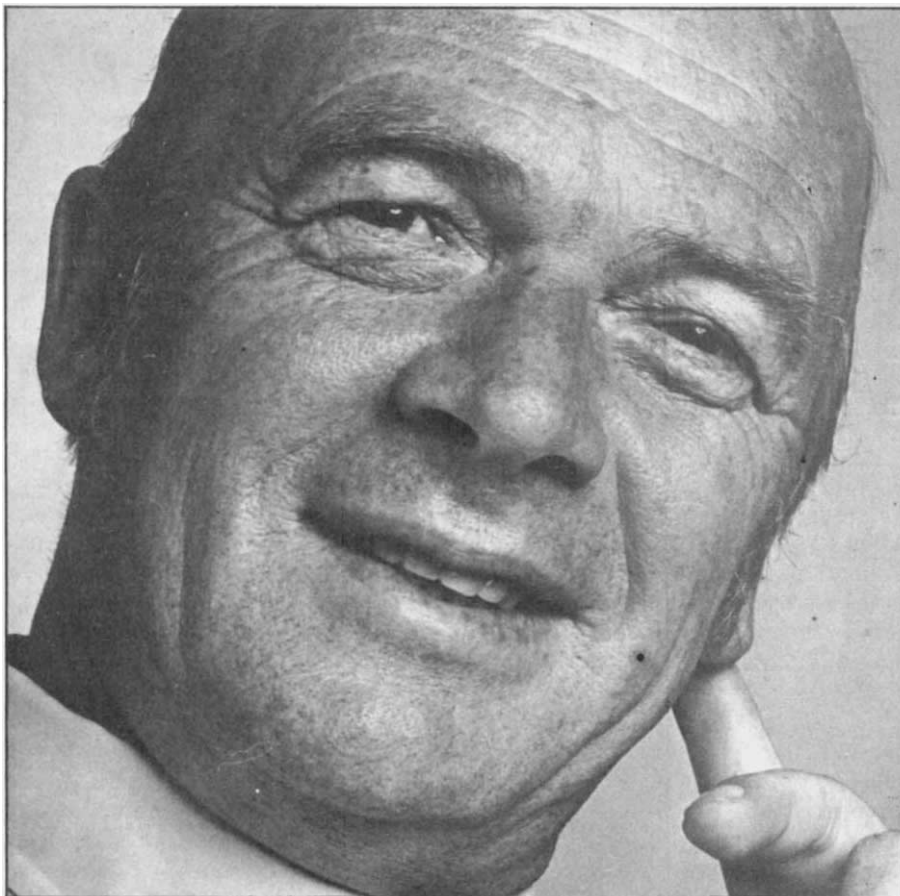
In a timely document published this week (*The Toxicity of Plutonium*, HMSO, 45p) the Medical Research Council (MRC), the body which advises the

government on radiation safety standards, taking into account the recommendations of the International Commission on Radiological Protection (ICRP), suggests that the part of the standard dealing with the inhalation of plutonium in insoluble form (for example, plutonium oxide, as used in the fuel of fast reactors) is too slack by a factor of five. The reasoning is based on the latest evidence available from the literature and on a reassessment of the 'critical organ' principle, which is still adopted for the plutonium standard by the ICRP, even though in the case of strontium-90 the commission has recognised that it is somewhat artificial. In essence, according to the principle, one regards a single organ or tissue as 'critical' in the sense that it is critically important in determining the MPAI (maximum permissible annual intake), at present set at 1 μg on the basis that the lung is the critical organ. The MRC points out that several different organs—bone, liver and gut—are in fact "irradiated to a similar level of risk", and that in those circumstances a reduction in the MPAI would be required. The way the MRC's calculations come out, three of the MPAIs (those covering the ingestion of soluble and insoluble plutonium compounds and the inhalation of soluble ones) are about right, whereas the MPAI for inhalation of insoluble compounds is rather off-beam.

In its report, which contains a wealth of detail on all aspects of the toxicity of plutonium, the MRC also considers the 'hot particle' thesis, according to which the local irradiation of the lung by very active insoluble particles is markedly more carcinogenic than irradiation when the same activity is uniformly distributed. Although the report is unimpressed with the contention of Tamplin and Cochran that the MPAI for inhaled insoluble plutonium is 115,000 times too large for particles above a certain activity, this is a point which needs to be cleared up once and for all by experimentation on animals which are better models of human beings than beagle dogs. If work with, say, primates reveals a greater susceptibility to plutonium, then clearly the safety standards will have to be looked at again, and urgently.

No part of the MRC's report, of course, has a direct bearing on the way in which the standards are enforced or the steps taken to minimise accidents involving plutonium. In the UK the Nuclear Installations Inspectorate, together with the National Radiological Protection Board, is charged with that job, and the Royal Commission on Environmental Pollution is taking an overview of the whole situation in its examination of radiation hazards in general.





Vice-Chancellor Perry: "experience can be adapted"

ments and to other types of education", and a spokesman for the university stressed that although it has concentrated on teaching to degree level, "the Consultancy Service believes that similar techniques and systems can be applied to other kinds of education and training, especially life-long learning".

So the university is already looking well beyond the usual narrow confines of higher education establishments. This kind of facility could clearly be of great use in keeping teachers up to date, providing training in the law, or perhaps as a means of welfare and hygiene training. And these developments show clearly how the Open University might be made, if not to pay its own way entirely, at least to contribute substantially to its own running costs. The present marketing activities of the university are far from insignificant, as the table shows, although it must be born in mind that the "margin" mentioned there is not entirely profit—there are other overheads involved in running the marketing operation. The global scale of this marketing operation is also surprising to anyone used to thinking of the Open University as a British establishment; only just over 50% of present net sales are in the UK.

Perhaps the greatest 'package' success of the Open University in the marketing field is the sale of complete courses to a few American colleges; in these packages books, course unit guides and broadcast material have been sold together and will be used just as they are in the Open University itself. This encourages speculation about the possibility of using modern communications facilities to centralise higher education to a great extent. If students in the USA can take what is in essence an Open University course (even, perhaps, an Open University degree) why should not the same facilities be used by UK universities? There is a powerful case to be made that, other considerations apart, the energy saving implicit in communicating a course to students, rather than transporting students to a course and housing them while they take the course, should alone lead to a major rethinking of our educational system. It is difficult to see that the interests of the country as a whole (or any country) would be less well served if the present scattering of scores of universities across the country were to be replaced by no more than half a dozen establishments organised along the lines of the Open University, perhaps on a regional basis. But that prospect is certainly not one which we are likely to see in the immediate future.

Any movement in that direction is bound to meet opposition from the academic establishment. But it is diffi-

Open for business

Britain's pioneering experiment in mass education, the Open University, has now developed to the point where it seems unlikely that any government will go so far as to close it down. This is a rather modest level of success—one would hardly be justified in saying that the Open University has in any sense 'come of age', even though it has produced its first graduates. But there was a real prospect that the incoming Conservative government of 1970 might have abandoned the experiment, and with higher education generally still in a parlous economic state the mere fact of the continued existence of the Open University is something to take note of. How might the bridgehead established by the Open University be used to best advantage in the coming years? And do conventional universities have something to learn from the Open University in terms of cost effectiveness?

—John Gribbin reports.

THE first lesson that might be learned by some academics is the combination of public relations ability and political awareness which the Open University staff seem to have. It's no secret that in the run up to the 1970 election the Open University's representatives took good care to lobby Margaret Thatcher, then in the Shadow Cabinet, and that this lobbying paid dividends when the Conservative government was elected, against all predictions, later that year. That might seem a wasteful diversion of effort which would, in an ideal world, be better spent on the development of the academic side of the university itself; but without that effort there might not

have been a university to develop.

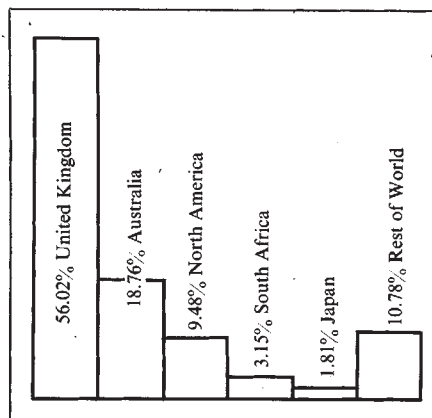
Late in 1974, the Open University revealed to the public gaze an example of its business acumen with the establishment of a consultancy service "to help interested bodies overseas establish similar techniques and systems" to the teaching-at-a-distance techniques pioneered by the Open University itself. At that time, Sir Walter Perry, Vice-Chancellor of the Open University, commented that "in four years, the university has shown that distance teaching can be effectively deployed to solve pressing educational problems... There is now enough evidence to justify the belief that our experience can be successfully adapted to other environ-

cult to counter the argument that the Open University approach is simply more efficient at getting its message across to large numbers of people at reasonable cost. The BBC runs just one studio for Open University programmes, at Alexandra Palace in London, and each television programme takes about one day of studio time to produce. There is also an outside broadcast unit, and radio programmes seem to be produced with hardly any effort at all. With about 50 producers at Alexandra Palace who have some kind of science qualification and this rather shoe-string operation the unit produces programmes which are used for five years before being replaced. And although the initial cost may seem high, the cost per student over that period must compare favourably with the cost per student of conventional lectures, even before account is taken of sales of programmes to other educational establishments.

In addition, for many of the science programmes the demonstrations provided in the Open University programmes are far more elaborate than anything which could be put on, and expected to work, under the restrictions of an ordinary lecture theatre. Again, an experiment which could not be financed by dozens of colleagues separately each academic year is a viable proposition when it only has to be put on once for the benefit of five years' intake of a much larger student body.

Even so, the total cost of the Alexandra Palace operation is not excessive even by the standards of some 'ordinary' universities, and looks even more reasonable when set against typical government expenditure on education and science. The total number of people now working in this BBC department is just over 300, and in 1973-74 the cost of the operation (wages, transmissions, filming, graphics, scenery, rental, rates, lines, administration, travel, processing and presentation all included) was £2,090,000. About half of this goes on wages, £600,000 for the actual manufacture of radio and television programmes (including all repeat fees), £250,000 for transmitter running costs and some £200,000 for premises (including lighting, heating and so on).

With a smaller proportion of pupils in secondary schools in Britain now wanting to stay on to take A levels, and fewer of those who qualify at that level wanting to continue their education at university level immediately afterwards, perhaps the time has come to rethink our approach to higher education. The arguments in favour of a break between school and university are well known, and perhaps the Open University approach offers the best route by which



O.U. sales, nine months to Sep, '74.

to return to the academic system for prospective students who had, temporarily at least, had enough of studying and examinations by the time they had reached A-level standard. Without yet going so far as to reduce the number of conventional universities drastically, it does seem that flexibility in combining Open University education with more conventional courses could make the university system as a whole more efficient, both in terms of cost and in terms of providing the greatest flexibility of courses for the greatest number of students.

The longer courses offered by conventional establishments are obvious possibilities for this kind of development; the time is not yet ripe for the Open University to offer a complete medical course (if only because of the difficulties in obtaining practical experience) but is there any real reason why all prospective medical students should not complete a 'remote teaching' pre-medical course before moving on to practical work? It is also difficult to see why law could not prove an ideal

course for the Open University treatment, if only the lawyers could be persuaded to move into the last quarter of the twentieth century. And if pre-medical courses are feasible, why not 'pre-university' courses, either as an option or as an essential prerequisite for all students who wish to take degrees? Two years of such study, say, would provide an excellent opportunity both for the prospective degree student to decide if he really does want to return to the academic fold and for the university of his choice to decide if they really want him. It would not be unreasonable for the work involved in such a two-year period of study to count as one third of a degree course, or, following the present Open University practice, for the same workload to be spread over three or four years. Then, the period of residence at traditional university need only be two years, with considerable advantages for the efficient running of those establishments. As most universities that run 'mature student' schemes can report, there would probably be great advantages in terms of a reduction in the drop-out rate and an improvement in academic standards.

There are imperfections in such a scheme, as there are in most, and it would hit hard at some entrenched positions. But with the present problems of the educational system (which have recently led the Secretary of State for Education and Science, Reg Prentice, to order what has been called "an inquiry on the reluctant students") something must not only be done but must be seen to be done. In the absence of other constructive proposals, this possible line of development of the Open University must surely be considered seriously. □

Open University marketing: ANALYSIS OF SALES FOR NINE MONTHS ENDED SEPTEMBER 30, 1974				
Item	Quantity	Value £	Cost £	Margin £
Book	168,149	177,347	67,710	109,637
Tape	3,550	20,642	6,840	13,802
Film	2,253	91,940	54,072	37,868
Home experiment kit	16	1,933	1,440	493
Colorimeter	45	1,026	828	198
Microscope	401	6,759	3,609	3,150
Sound level indicator	245	5,435	1,813	3,622
Logic tutor kit	97	1,983	839	1,144
Analogue control kit	45	586	256	330
Power supply meter	34	664	383	281
Bobcat	103	537	304	233
Gramophone record	221	228	110	118
		309,080	138,204	170,876
Other income				
Royalties—Harper and Row		8,000	—	8,000
Other		4,070	—	4,070
Film library		8,702	1,803	6,899
Recording licences		2,810	—	2,810
		332,662	140,007	192,655
Sources of total sales:				
UK		£181,000		
Overseas		£152,000		
		£333,000		

international news

THE economic theories of John Maynard Keynes, coupled with the rising price of oil and the insatiable demands of the Pentagon, have helped produce promises of massive increases in federal support for science and technology in the United States. That, at least, is the prospect held out by President Ford's budget proposals for the 1976 fiscal year, which were unveiled earlier this week.

Taken at face value, the budget anticipates that the federal government will set aside some \$21,600 million for research and development next year—a huge increase amounting to \$2,822 million above the expected support for science and technology this year. At 15.5%, the increase would be more than enough to keep pace with inflation and if it is all spent, it would represent much the biggest increase in the science budget since the Second World War.

Unfortunately, however, the figures cannot be taken at face value and should be treated with extreme caution. For one thing, they will almost certainly be altered by Congress and, for another, the picture is not quite as rosy as it seems at first sight.

The President's budget is a remarkable document. Hundreds of pages of facts and figures richly laced with rhetoric, it is the product of thousands of man-years of effort and as such it is the most important policy document produced by the Administration each year.

The sharp increases which have been proposed for research and development next year owe their existence to three chief factors. The first is a massive increase in the budget proposed for military science and technology. The second is another sharp upturn in proposed

US budget: energy and the military do well

from Colin Norman, Washington

spending on energy research and development, and the third is the fact that President Ford, like his predecessor, has been converted to the Keynesian view that an expanding federal budget is a useful weapon against recession. Ford's total budget would entail outlays of \$350,000 million and a budget deficit of nearly \$52,000 million.

● A total of \$11,400 million has been proposed for military research and development, which represents a staggering increase of \$1,900 million over anticipated expenditures this year. The budget document is characteristically vague about how this increase in funds will be spent, but the Trident submarine, the B-1 strategic bomber and a project to provide "options for possible future deployment of a new intercontinental ballistic missile system" are among the items mentioned. In addition, a large increase has been proposed in a programme for testing nuclear weapons, the objective being to complete all large-scale testing before the introduction of a possible threshold test ban. Some \$201 million has been requested for nuclear testing.

The Department of Defense's share of the proposed military research and development budget amounts to some \$10,600 million, of which just over \$2,000 million is classed as research.

● Energy research and development is set to receive an increase of nearly

\$200 million, to reach a total of \$2,360 million next year. (See table).

Although nuclear fission would get the bulk of those proposed funds, and the liquid metal fast breeder reactor alone is set to receive nearly \$500 million, massive increases have been proposed for solar, geothermal, fusion and coal conversion technologies. The Energy Research and Development Administration have also announced plans to build a large Tokamak fast reactor at Princeton University in 1977. Designed to burn deuterium and tritium the machine would be the first fusion fast reactor in the US and it is expected to cost \$215 million.

● The Administration has proposed an increase of nearly \$300 million in the budget of the National Aeronautics and Space Administration, chiefly for the development of the Space Shuttle.

Simple addition shows that between them, those three areas carry off all but about \$480 million of the entire increase proposed for the federal science and technology budget. Some programmes have therefore been squeezed.

Biomedical research is one such area, and space research is another. No new space project will be started by NASA next year, and aside from development of the Shuttle the budget for most NASA activities will be held constant. Sufficient money has, however, been proposed to keep all existing space projects alive, and NASA has also been allowed to proceed with plans for a third in the series of Earth resources technology satellites.

Nevertheless, the overall picture for science and technology is remarkably bright. According to an analysis prepared by the Federal Council on Science and Technology, the budget would entail an increase of about 8% in expenditures on basic research, thereby reversing the trend of declining support of such activities which began in the early 1970s. Between 1970 and 1974, for example, federal outlays for basic research declined by 15% in purchasing power.

Another indicator of proposed support for basic research is the fact that the budget for the National Science Foundation (NSF) is set for a healthy increase. Overall, the Administration has proposed that the NSF's budget should increase by \$78.3 million next year, to reach a total of \$775.4 million. Some 83% of the NSF's budget is earmarked for support of basic research. The NSF's support through individual

Federal energy research and development programme
(\$ million)

Programme area	1974	Obligations 1975*	1976*
Direct energy research and development:			
Fossil energy	110	435	440
Solar and geothermal	45	102	123
Conservation	39	86	88
Nuclear energy	756	942	1,102
Environmental control	66	103	83
Total direct	1,016	1,669	1,837
Support programme:			
Environmental effects	138	264	273
Basic research	175	233	250
Total support	313	497	523

*Estimate.

grants, for example, is set to increase by almost \$40 million, to reach \$380 million.

As for the support of research and development in colleges and universities, an analysis provided by the Office of Management and Budget indicates that academic scientists will receive only about \$2,278 million of the total federal research budget proposed for next year. That represents an increase of only about \$52 million, which will not keep pace with inflation.

How will Congress treat the budget proposals? First, it should be noted that Congress this year has an entirely new mechanism for dealing with the federal budget. In the past, the Administration's budget proposals have been chewed over by individual appropriations committees in the House and the Senate, each of which is responsible for specific departments of the federal government. Since each committee operates independently, the process has been likened to each committee writing cheques without regard to what is in the bank.

This year, however, budget committees in the House and the Senate will establish an overall budget ceiling, and decide maximum amounts for each appropriations subcommittee to work to. Thus, for the first time, Congress will be able to compare directly the worthiness of programmes supported, for example, by NASA with those supported by the Department of Health, Education and Welfare.

Nevertheless, congress is unlikely to reduce budgets for such politically sensitive areas as energy research and development—although it may well reorder priorities within the energy research budget to favour environmental research, and solar energy and coal research, as against nuclear power. Similarly, the money available for biomedical research will probably be increased above the Administration's budget request.

Finally, it should be noted that two years ago, when Mr Nixon announced that he no longer required a full-time Science Adviser, the scientific community suffered a fit of apprehension that the federal science budget would immediately show a precipitous decline. But Dr H. Guyford Stever, who inherited some of the Science Adviser's responsibilities, noted last week that "some people have said that federal science has been drifting. I am pleased to note that it has been drifting upwards".

● During the past few years the National Institutes of Health (NIH) and its clients in the universities and medical schools have been awash with turmoil and indignation over the Administration's policies for supporting biomedical research. The budget un-

veiled this week will not help much in calming the troubled waters.

To begin with, the Administration has proposed cutting some \$315 million from the funds that Congress has already voted for the NIH for the 1975 fiscal year (which is now more than half over). Then, the budget proposed for the NIH in 1976 would reinstate only \$72 million of that cutback, to give the agency a total of \$1,805 million—\$279 million less than Congress has approved for 1975. To add insult to injury, the Administration is, naturally enough, claiming credit for proposing a \$72 million increase for NIH next year.

Another controversial item in the budget is that the Administration has signalled its intention to reduce expenditures on biomedical training. Attempts to do that in the past have invariably brought the medical schools to simmering point.

Although Congress will not go along with all the Administration's proposals for the NIH, another long and bitter battle can be anticipated and biomedical scientists will have to wait to see exactly how much money the federal government intends to spend on health research over the next 18 months.

As far as the NIH's budget for this year is concerned, the problem lies with an appropriations bill which Congress finally passed in December last year. Although the bill exceeded the funding levels recommended earlier by President Ford for a number of programmes, Ford signed the measure into law with the understanding that he would later ask Congress to revise it. His proposed revisions include reducing the total which Congress appropriated for the National Cancer Institute by \$128 million, and similarly taking \$38 million from the budget of the National Heart and Lung Institute and axing a total of \$190 million from the budgets of the other eight institutes. Furthermore, he has proposed slicing \$104 million from the funds that Congress has appropriated for programmes dealing with alcoholism, drug abuse and mental health.

Congress must, however, give its express approval within 45 days before any of those reductions can be made, and it is not likely to do so. For one thing, Congress raised up the NIH's budget in December in spite of an urgent plea from Ford to reduce it. And for another, during the past four years Congress has consistently appropriated much more money for biomedical research than the Administration has wanted to spend. It is conceivable, however, that, as a token gesture, Congress may shave a little off the NIH's budget for this year just to show willing to hold the line on federal expenditure.

To put the matter in perspective,

however, it should be noted that even with the proposed revisions in the NIH's 1975 budget, the agency would still have some \$265 million more to spend this year than last.

Thus, with budgets for the 1975 fiscal year far from settled, the Administration's proposals for next year are totally unreliable as a guide to what will actually be spent. Nevertheless, for what it is worth, the Administration has proposed a budget of \$605 million for the National Cancer Institute and \$293 million for the National Heart and Lung Institute. Those proposals would represent increases of \$36 million and \$7 million respectively over the revised budgets of the two agencies, neither of which is sufficient to keep pace with inflation, and both of which are well below the budgets approved by Congress for this year. A similar picture can be painted for all the other institutes of NIH.

As for biomedical training, the Administration has proposed that the NIH should allocate some \$124 million to training grants next year, compared with \$131 million this year. That proposal is unlikely, however, to find much sympathy either in Congress or in the medical schools, for last summer Congress passed a bill which authorised expenditures of some \$208 million a year on biomedical training. Congress approved that measure in response to previous attempts by the Administration to do away with the NIH's training programme entirely—attempts which threw the biomedical research community into apoplexy.

Thus, the federal government's support of health research is again marked by considerable confusion and is likely to become a source of yet more controversy. But two factors may help to clarify the situation in the next few months.

First, Senator Edward M. Kennedy has announced that he will carry out a thorough investigation of the federal government's biomedical research policies this year through his Senate Health Subcommittee. Although Kennedy has usually supported the biomedical research community's complaints against the government, he served notice in a speech at Yale University last month that the medical colleges have often failed to examine critically their most cherished programmes. And last week, President Ford finally appointed five members of a special commission, set up by an Act of Congress, which will undertake a review of the NIH's policies and programmes. The review will take 18 months and co-chairmen of the panel are Dr Franklin Murphy, chairman of the board of the *Los Angeles Times*, and Dr Robert Ebert of Harvard University. □

AN OPEN letter from the Soviet dissident Dr Andrei Sakharov to the head of KGB, Mr Y. V. Andropov, has just reached the West. The letter (shortened slightly in this version) reads as follows:

'On December 20, I received a letter from a mythical Russian Christian Party, which contained threats directed at my son-in-law, Efrem Yankelevich, and my one-year-old grandson.

The authors of the letter threaten to destroy them, if I continue my civic activity.

The content and whole tone of this letter make it obvious that it was composed by your officials with the aim of frightening me and forcing me to keep quiet.

The previous day I had received notification from the visa office that Yankelevich and his wife had been refused permission for a journey to the United States at the invitation of the President of the Massachusetts Institute of Technology.

Prior to this, their application had lain unanswered for a year and eight months. This coincidence cannot be accidental. I declare that the members of my family are hostages and are being used as a means of exerting pressure on me.

This has been confirmed again today. Two of your officials, who were tailing my son-in-law on the street, repeated word for word the same threats as before (adding some foul language) and demanding an end to my activity.

I demand an end to the harassment of me. I demand an end to the thugery, and assurances of security for my family.

Don't disgrace your department even more by threatening the lives of children in the way practised in Stalin's time.

In the present circumstances, for which your department bears the responsibility, I demand that permission be granted at once to Efrem Yankelevich, his wife Tanya Semyonova and their son to travel to the US for an unlimited period, but on Soviet passports so that they will have the right to return home when their security can once again be assured.

It is quite clear that they are hostages. The only way of ending this form of pressure on me is to give them visas at once.

I also demand immediate permission for my wife to travel to Italy for treatment (for her blindness).

I demand an end to the disconnecting of my international phone calls.

I demand an end to the judicial and extra-judicial persecution of my friends, including Sergei Kovalov, who was arrested on 27 December.

At the same time as sending you this letter I am making it generally available and appealing to the world public for its support and defence.'

Vera Rich adds:

With the enforced departure of Solzhenitsyn and Medvedev, the Academician Andrei Sakharov, has found himself in an increasingly exposed and lonely position as Russia's "voice of conscience". That the Soviet authorities would like to silence him was only too apparent from the vicious press campaign launched against him in September 1973. This having failed, the alternatives remaining are exile or

Dissident voices



Polskii: expert *refusnik*

physical violence—directed not at himself but at his family—his wife, step-daughter and her husband and baby.

● Physicist Viktor Polskii, who, after more than four years' delay, was finally allowed to leave the Soviet Union for Israel in December 1974, is perhaps one of the best authorities on the problems facing the "refusnik", since in his case, the customary harassment included a charge of dangerous driving, which could have carried a considerable prison sentence, but which, under the pressure of world opinion finally resulted in a fine of 100 roubles (£50).

Talking to *Nature* this week about Jewish emigration, and, in particular, the emigration of Jewish scientists from the Soviet Union as a result of the recent rejection of the trade agreement, Polskii stressed that in fact the policy of permitting limited emigration to Israel was begun before the Soviets entered upon their course of *detente* and trade agreements with the USA. Emigration, or rather, the appli-

cation for emigration, began on a large scale in 1970-71, long before the top-level trade talks began. He says that the idea of limited emigration was decided at the Politbureau meetings held in preparation for the Twenty-fourth Party Congress which opened on March 26, 1971. (A large party of emigrants left on March 1st). It would seem, however, that the scale of the applications somewhat disconcerted the Soviet authorities, resulting in the imposition for a time of the "education tax" on Jewish intellectuals wishing to emigrate, together with the policy of harassment and general unpleasantness which persists to this day.

For the future, Polskii sees the continuation of "limited" emigration, but with increasing pressure on applicants, especially intellectuals. The five cities of Moscow, Leningrad, Kiev, Khar'kov and Minsk together account for 1,000,000 Jews—some 30% of the Jewish population of the Soviet Union, and virtually the entire Jewish intelligentsia. It is from these cities, that he expects emigration to be most difficult. Further, there is the concept of the "closed town", which has been mentioned in visa refusals—towns such as Sverdlovsk, from which, it appears, no emigration is to be permitted at all.

The current pressure on *refusniks*—dismissal from one's job, followed by prosecution for parasitism (being without visible means of support), may well account for a certain falling off in the number of applications. Nevertheless, says Polskii, the would-be emigrants are not entirely abandoning hope. While not actually putting in a visa application at present, a great number of them are careful to keep themselves provided with an up-to-date invitation from a relative in Israel, so as to be ready to apply the moment circumstances seem slightly more propitious.

● The Russian biologist and writer Vladimir Konstantinovich Bukovskii, at present serving a twelve-year prison sentence is a result of supplying Western psychiatrists with documents on the confinement of dissidents in mental institutions is reported to be dangerously ill. Although suffering from a heart condition related to rheumatism, a liver condition, and duodenal ulcers, Bukovskii is receiving no medical attention, and medicines sent to him by his mother have been returned. He is unable to digest the prison diet of salt herring, spratts and cabbage. In an appeal to Minister of the Interior Sholokov, sent on January 29, Nina Bukovskaya pointed out that her son "is being slowly murdered by being deprived of medical treatment and correct medical diet". To date, no reply to her appeal has been received.

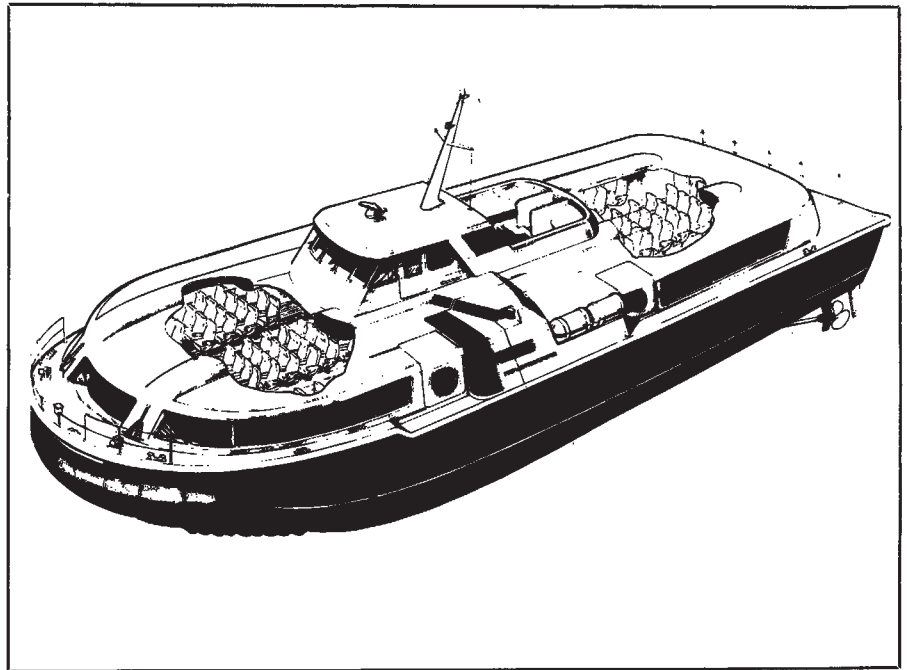
Making money and influencing people

from Angela Croome

LAST week the UK National Research Development Corporation (NRDC) announced that it was putting slightly over £500,000 into the development of a 200-passenger sidewall hover-ferry, the Hovermarine 5 (HM5). This caused some raised eyebrows, as Hovermarine, managed by Britons with a factory at Southampton, is now an American-owned company and the NRDC is, as its name suggests, dedicated to the promotion of inventions and applications to the national benefit using government money.

The loan is not anomalous, however, though it is of more than average interest. The NRDC holds the key hovercraft patents and issues the licenses for using them worldwide, so that any expansion by their licensees (such as Hovermarine) brings cash flowing in to London. (The NRDC itself has made a profit for the last few years.) In fact under its new American-style management Hovermarine's craft are now more widely sold than any other hover-ferries in the world, with 30 HM2s operating in 14 different countries, and the business has consistently doubled each year since the 1969 takeover. The company has played down the "new technology" aspect of the product and has emphasised its solid commercial and environmental advantages—such as its high speed, low cost, wake-free passage and high manoeuvrability. Currently, Hovermarine is the only licensee for sidewall hovercraft. Sidewalls, which provide solid containment for the air cushion along the craft's long axis and, acting as twin keels, aid manoeuvrability, essentially economise on fuel and power compared with competitive craft such as the fully amphibious hovercraft, the motorboat and the hydrofoil. The HM2 cruises at 30 knots; the HM5 is expected to cruise at 45 knots. By putting up the most substantial sum it has spent on hovercraft for a number of years the NRDC is saying in unequivocal fashion that it is pinning much faith in the future of sidewall hovercraft as sheltered-water ferries and, by implication, approving the commercial and sales skills of its licensee every bit as much as its technical flair.

The NRDC has just passed its 25th anniversary and has also gained a new Managing Director, Bill Makinson; he is not new to the corporation, however, having worked there since the mid-1960s. Does the major new loan reflect a shift in policy with a change of team, or the fruit of a quarter of a century's experience in seeking out and fostering worthwhile innovations?



The Hovermarine 5: £500,000 development programme

On the occasion of the 25th anniversary and the announcement of the annual accounts, the NRDC revealed that it was being heavily underused and described efforts to rectify the position. It is entitled to borrow £50 million from the government for encouraging promising inventions and techniques. Only £21 million is at present in use. The board is concerned that the existence of the corporation, much less its role and method of operation, is scarcely known to the part of the population that could benefit from it. Government research establishments have provided a steady stream of ideas for NRDC sponsorship in the past. These are now being deflected to some degree, following the rearrangements resulting from the Rothschild customer-contractor principle. Only 2% of private inventions that reach the corporation are worth following up. Few small and medium businesses—a major untapped source of innovative concepts—seem to be aware of the NRDC's existence. Good ideas from the universities are even rarer and this is attributed again to ignorance of the existence of the corporation. The NRDC has over the past couple of years found that there is not only a total shrinkage of good proposals coming its way but that the balance is poor. It likes to keep a reasonable match between its nine groupings.

In the face of this surprising situation in a tight money market, the corporation has launched an advertising campaign to get its opportunities better known. It has also set aside £1 million specifically for investment in good ideas from the universities. There is no indication that either move has had much impact.

There are various criticisms that can be levelled at the NRDC's present mode of operation and which may well be bound up with its faceless image. Just how it runs, and on what basis decisions are reached, are obscure. Just how much interference it experiences or fears from government departments is an open question. For instance is it really likely that a proposal turned down by Science Research Council would be pursued by the NRDC—the Laithwaite 'magnetic river' project is a case in point. The corporation claims that Whitehall has never gibbed at one of its projects; but is that necessarily a matter for congratulations? The virtue of such a body is that it should provide a genuinely alternative option. There is a widespread sense that the corporation operates too much as a bank and insufficiently as a sponsor of research and development as its title suggests it should. It is free to encourage and invest beyond the development phase of bringing a good product to the market place, and it is now generally acknowledged that the major costs arise at the production and marketing stage. But there are few, if any, cases where the NRDC has helped over these final and most costly hurdles, and plenty of examples of products that have failed because of lack of capital or expertise at the end of the 'run'.

There is no lack of expertise, skill and sound judgment in NRDC. It just does not seem to make the most of itself and under present economic conditions there is surely more need of such a supporting body than there ever was. The stocktaking at the start of its second quarter century seems an excellent moment for reassessment and review. □

THE underlying purpose of the annual meeting of the American Association for the Advancement of Science (AAAS) is not all that easy to pin down. Partly an attempt to improve that ill-defined entity the public understanding of science, and partly a forum for exchanging views on a variety of social and political issues, it is, in the words of the retiring AAAS President, Roger Revelle, a "scientific smorgasbord". Whatever its *raison d'être*, the 141st such event took place last week in New York City and a good time was had by all.

The theme of the meeting was "Science and the Quality of Life", which was interpreted broadly enough to include a session on the emotional reactions of plants and a talk by Isaac Asimov on "The Science Fiction Writer as Prophet". Discussions concerning the health effects of pollutants, the energy crisis, world food shortages and urban technology constituted a large part of the programme and there was even the occasional session devoted to scientific research.

One notable feature of the meeting was that few of the sessions were devoted to discussion of scientific results, undiluted—or unenriched as the case may be—by considerations of public policy. That aspect reflects the desire on the part of the organisers to make the annual AAAS meeting more concerned with the interactions of science and society. It also reflects the fact that few scientists think of saving up their research results to announce them at the AAAS meeting—more specialised gatherings and quick-publication journals now fulfil that role.

● With Vice-President Rockefeller engaged in a study of how science advice should be plugged into presidential decisions, a session devoted to the question "Does the President still need a science adviser?" may seem like the height of irrelevance. But it turned out to be highly pertinent. Although only one member of a panel, consisting chiefly of luminaries from the world of science policy, ventured the opinion that the President has no need for a science policy office at his elbow, there turned out to be a huge spread of opinion on what the mechanism should be, and on what it should do.

Before the meeting took place, a few of the participants held a press conference at which two former officials in the Nixon White House confirmed what most people had suspected—that Nixon abolished the post of science adviser because he disagreed with much of the advice he was getting. Dr Edward David, Nixon's last science adviser, spoke of a "falling out"

between the President and the scientific community and said that "there definitely was a perception in the White House that the science advisers were using their expertise to grind axes". And Clay Whitehead, former Director of the White House Office of Telecommunications Policy, said that White House officials felt that the science adviser showed more allegiance to the scientific community than to the Administration.

New York diary

from Colin Norman

Most members of the panel thought that an office to provide policy analysis and advice on issues involving science and technology should be relocated near the centre of power. Franklin Long, of Cornell University, a former member of the President's Science Advisory Committee (which Nixon also abolished), favoured a three-member council of science and technology advisers. David also argued for a science policy office, but warned that it should be established in such a way that it does not upset the existing (and little noted) arrangements for getting science advice into the National Security Council and the Domestic Council. But George Reedy, former press secretary to President Johnson, argued that a full-time science adviser is not needed and that an *ad hoc* arrangement to provide scientific analysis of specific issues for the White House would be a better arrangement.

● A little light entertainment was provided later in the week by a debate—if a piece of comic opera can be so termed—over whether or not plants have been shown to respond emotionally to external stimuli. The debate took place between a number of botanists and Mr Cleve Backster, a polygraph expert and former CIA employee, who carried out an experiment in 1966 which, he claims, shows that plants respond electrically to human thoughts and emotions. That experiment has since turned up as the foundation of a best-selling book, the *Secret Life of Plants*, and resulted in widespread popular belief in the United States that plants have emotions.

Backster, an ebullient man who displayed a strong tendency to lead with his chin, failed to convince many of his critics that his experiment demonstrated anything other than poor scientific method, while two scientists who had tried unsuccessfully to repeat Backster's amazing work, failed to shake the belief of about half of the

audience in the emotional capacity of plants.

The crucial experiment, published in the Winter 1968 issue of the *International Journal of Parapsychology*, was carried out in Backster's New York laboratory. It was an attempt, in short, to see whether plants respond electrically to the termination of animal life.

He hooked three plants up to polygraphs, by sandwiching one of their leaves between electrodes, and monitored their electrical activity when brine shrimp were killed in a separate room. The shrimp were killed (terminated, Backster says in his paper), by being dumped into a bath of simmering water. Amazingly enough, the polygraph chart registered electrical responses similar to those which show up when human emotional reactions are monitored, and the results were statistically highly significant.

But two other scientists related their fruitless attempts to repeat Backster's work. Edgar L. Gasteiger, of Cornell University, told how experiments conducted over two years by three of his students "with many types of control that Cleve Backster did not use" failed to show any plant response to the killing of brine shrimp. The other would-be repeater of Backster's results was John Kmetz of the Science Unlimited Research Foundation—a body which Backster himself helped to establish. Kmetz used a total of 42 plants, 84 shrimp drops and 84 control drops using sterile water. His plants failed to react.

Although he has not repeated his original experiment, Backster, undeterred, claims to have achieved even more spectacular results with the bacteria in yoghurt. He claimed that when he added milk to one yoghurt culture, another culture across the laboratory which was hooked up to a polygraph gave off electrical signals. That piece of information, not surprisingly, got widespread attention from the press, for, as one journalist put it, "whose editor can resist a story about yoghurt that talks?"

Perhaps the chief question raised by the session was why was it held. Dr Arthur Galston, of Yale University, who arranged it, said that a poll of his students turned up the finding that about half of them talk to their plants, and about half of that group believes that it does some good. "There is a gap", he said, "between what scientists believe and what the lay public believes", and it is important to give the issue an airing. It must be the first time that an entire session of the AAAS has been devoted to a single experiment which has not been repeated in eight years, even by its originator.

news and views

RECENTLY our knowledge of the recipes which galaxies might use for cooking up heavy elements has increased rapidly. In an article in the *Astrophysical Journal* (193, 327; 1974) Peimbert and Torres-Peimbert report the detection of a difference of helium abundance between the interstellar gas in our own Galaxy, as observed in the Orion and η Carina nebulae, and ionised gas regions in the Large Magellanic Cloud, one of the nearest neighbours to our Galaxy. The difference is small (about 20%), but this is the first time a difference has been detected which is significantly greater than the observational and interpretive errors. Although the bulk of helium in the Universe is thought to have been synthesised in the big bang, further enrichment with helium comes through the nuclear conversion of hydrogen as the primary energy source for stars. The low helium abundance observed in the Large Magellanic Cloud implies that the gas in that galaxy has not been enriched as much as that in our own Galaxy. The present conditions in the Universe suggest that the big bang would have produced isotopes of hydrogen and helium, but no metals. The metals (which to an astronomer are anything heavier than helium) come from thermonuclear reactions during supernova explosions of stars which die with a bang rather than a whimper, the metals observed in the interstellar medium probably originating from stars with mass of more than ten solar masses. The low helium abundance of the Large Magellanic Cloud fits in very well with the observed low metal abundance for the same system—again this shows less enrichment by material processed in stars.

The determining factor in the amount of enrichment that takes place almost certainly involves the ratio of mass in stars to the mass in gas which is set up during the early fragmentation (or perhaps agglomeration?), collapse and evolution of a protogalaxy into a galaxy from the debris of the big bang. The rather irregular Magellanic Clouds have considerably more gas than our spiral Galaxy, in accord with the general observation of a rough inverse correlation between the metal abundance and gas content of galaxies. Just when and how many stars form (perhaps determined by the amount of angular momentum present), together with how much gas is left, will not only affect chemical evolution but may

Galactic cookery

from M. G. Edmunds

also determine the morphological type of the galaxy on the Hubble sequence—the reason why chemical evolution is of interest to all studies of evolution and dynamics of galaxies.

A first approach to models of chemical evolution, examined in some detail by Tinsley (*Astrophys. J.*, 192, 629; 1974), comes through observation of stars in our own Galaxy. It has been known for many years that the simple idea of stars forming, evolving and exploding at a uniform rate is inadequate. The metal abundances of stars can be deduced from spectroscopic analysis and their ages deduced by comparing their spectroscopic and photometric properties with theoretical stellar evolution models. The resulting relation of metal abundance with age shows puzzling features. Enrichment in metals has certainly not been uniform, but seems to rise rapidly during the early part of the Galactic life and to have increased only very slowly since then. Even some of the oldest stars seem to be metal rich. A second problem occurs because it is observed that the number of metal-poor dwarf stars is far too small compared with the number of metal-rich dwarfs to be explained by uniform enrichment. So what really happened? A popular proposal is that early on (perhaps even before the Galaxy had formed) the relative number of high mass stars to low mass stars formed was greater than for recent star formation. This shift in mass function, caused either by forming more massive stars or fewer less massive stars, implies more supernova metal synthesis per mass of gas converted into stars, and a rapid build-up of elements with only a few dwarfs formed before the Galactic gas became quite rich in metals. A change in the initial mass function for star formation is not as arbitrary as it might seem because the initially low metal abundance could radically alter both the cooling of the gas required for protostellar collapse and the mechanisms which limit the greatest mass which can condense into a star. The biggest uncertainty in all

models of galactic evolution arises because we just do not have a reliable theory of star formation—we have no way of predicting even whether a low metal abundance would shift the mass function towards higher or lower masses.

Playing around with the star formation in a rather different way has produced the attractive “metal-enhanced star formation” model of Talbot and Arnett (the latest in a series of papers appeared in *Astrophys. J.*, 190, 605; 1974). They propose that star formation at a given time only occurs in regions of gas which are more metal-rich than average, although there is some uncertainty whether or not large enough inhomogeneities in the metal abundance of the interstellar medium can be set up. Metals are preferentially incorporated into stars, the model accounts for the observed abundance versus time variations and the metal-poor dwarf problem, and has the additional attraction that a constant initial mass function is perfectly adequate. If this model is correct, it is telling us interesting things about star formation—the requirement for high metals and an invariable mass function.

In external galaxies, and probably our own, another phenomenon requires explanation. The abundance of metals is greater near the centre of a galaxy than farther out. How were such abundance gradients set up? Larson (*Mon. Not. R. astr. Soc.*, 166, 585; 1974) and Larson and Tinsley (*Astrophys. J.* 192, 293; 1974) have investigated numerically the collapse of a spherical protogalactic gas cloud with various boundary conditions, and simple assumptions about the conditions for star formation. Some of these models reproduce well the time variation of metal abundance, and also set up abundance gradients by the fall of gas, enriched by supernova explosions, towards the centre of the galaxy. Another feature is the prediction of an early peak in star formation and explosion which Larson suggests might trigger the onset of Seyfert or QSO behaviour in some massive galaxies. This has attractions as it suggests a quiescent ‘normal’ evolution of a QSO until it becomes superluminous, and would allow the build-up during the normal evolution of the observed (albeit rather uncertainly!) approximately solar abundance of metals in quasar spectra. In his most recent paper (*Mon. Not. R.*

astr. Soc., **169**, 229; 1974) Larson adds a new refinement to his models in the effect of the energy input of the supernovae on the galactic gas. This energy tends to blow out of the galaxy the metal enriched gas as it falls towards the centre, the amount expelled depending on the escape velocity and hence the mass of the galaxy. This offers an explanation of the apparent correlation of the metal abundance of a galaxy with its absolute magnitude, from several times less than solar abundance for dwarf ellipticals with low escape velocities to a few times more than solar for massive ellipticals.

A rather imposing blend of an initial formation model and a collapse-infall model is being proposed by Ostriker and Thuan. From other arguments on galactic rotation curves and stability of galactic disks, Ostriker and co-workers have proposed that spiral galaxies have massive stellar holes containing perhaps up to ten times the mass of the disk. During initial collapse star formation is rapid, and the en-

riched gas expelled from massive stars falls to form the disk. The longer lived dwarf stars persist in the halo, sufficiently faint to escape visual detection, but still contribute a significant infall of material into the disk by mass loss from their surfaces. For all models the realistic inclusion of such effects as violent events in the nuclei of galaxies, sporadic star formation and radial gas flows is yet to come. But already we have the problem of non-uniqueness of models which will only be resolved by tighter theoretical and observational constraints. For example the useful limits on the amount of gas cycled through star formation from deuterium abundances (which is destroyed by passage through stars) can be used once it is clear whether or not the big bang was the only source of deuterium.

It seems that present galactic chemical evolution models are similar to culinary ones—they may vary greatly in their ingredients, but with judicious stirring a palatable cake may result from many of them.

Absorption maxima

It is this interaction that gives the rhodopsin molecule its unique spectroscopic properties. All known creatures with image-forming visual systems employ a rhodopsin-type visual pigment, and the absorption maxima of these pigments lie over a colossal range of wavelengths throughout the visible and near-ultraviolet regions. The gamut of rhodopsins has been extended down to 345 nm by the confirmation by Paulsen and Schwemer (*Biochim. biophys. Acta*, **283**, 520; 1972) that the pigment of the neuropteran, *Ascalaphus macaronius*, is a true rhodopsin. The retinal-based pigment of longest wavelength is that of the bronze turkey (*Meleagris* sp.), with a maximum at 562 nm. Thus any theory of pigment absorption must explain how the absorption maximum of retinal at about 380 nm can be either blue-shifted by 35 nm or red-shifted by up to 182 nm. Various theoretical models have been put forward in the past, but the most popular at present is based on a single covalent bond—a protonated aldimine (Schiff's base) linkage—between retinal and an ϵ -lysine group on the protein. This will cause the retinal absorption maximum to be red-shifted by up to 100 nm, and the fine tuning required to give the absorption maxima of different species is then achieved by secondary interactions between the chromophoric group and other amino acids. This can be pictured in its simplest form as the effect of a single negative charge fixed at a certain point near the polyene chain. Recent detailed molecular orbital calculations show that as this counter-charge is moved away from the positive charge at the aldimine bond, the absorption maximum goes to longer wavelengths, approaching a limiting value similar to that found in nature (Waleh and Ingraham, *Archs Biochem. Biophys.*, **156**, 261; 1973; Suzuki, Komatsu and Kitajima, *J. Phys. Soc. Japan*, **37**, 177; 1974).

Retinal can form a number of geometrical isomers, but only one of these, the 11-*cis*-isomer, will react with bleached rhodopsin to regenerate the original pigment. For this reason, it has been assumed that the conformation of the chromophoric group in the binding site is 11-*cis*, and that the shape of the molecule is the same as is found in crystalline 11-*cis*-retinal. Gillardi, Karle and Karle (*Acta Cryst.*, **28B**, 2605; 1972) showed that crystalline 11-*cis*-retinal is not planar like the other isomers, but is twisted and should thus be optically active. The intact rhodopsin molecule shows a circular dichroism associated with the long wavelength absorption band that is not seen in solutions of 11-*cis*-retinal. Honig, Kahn and Ebrey (*Biochemistry*, **12**, 1637;

The enigmatic protein

from Aubrey Knowles

It is nearly 100 years since Kuhne extracted a pink, light-sensitive material from the retinas of a number of animals. He realised that these substances—later called rhodopsins—were proteins and that they were responsible for the conversion of light into neural signals. Since then, a great deal of effort has been made to understand the rhodopsin molecule, but its structure is as mysterious as ever. This unique molecule is thermally quite stable, yet will undergo major chemical and structural changes on the absorption of a single quantum of visible light: beyond acting as a transducer of light, it is also the principal structural protein of the photoreceptor cell membranes.

This last factor has hampered investigation by the usual techniques of protein chemistry, for rhodopsin is a hydrophobic molecule and is totally insoluble—thus it cannot be crystallised. Until recently, most investigations have been carried out on detergent extracts, and the results of these are of questionable validity for all detergents denature the molecule to some extent. The detergent-extracted molecule has been found to contain, beyond the protein part, phospholipid, carbohydrate and a polyene called retinal. The latter is the 'chromophoric group' that gives the molecule its colour: exposure to light causes the chromophoric group to break away from its binding site, and the colour is lost. The supposition that the binding site is on

the protein part of the molecule has recently been confirmed, for nearly all of the phospholipid can be removed enzymatically without loss of colour (Borggreven, Daemen and Bonting, *Archs Biochem. Biophys.*, **151**, 1; 1972). It has also been found that up to 40% of the protein can be removed by proteolysis without causing bleaching (Bonting, de Grip, Rotmans and Daemen, *Expl Eye Res.*, **18**, 77; 1974; Trayhurn, Mandel and Virmaux, *FEBS Lett.*, **38**, 351; 1974; Saari, *J. Cell Biol.*, **63**, 480; 1974).

In 1968, Heller (*Biochemistry*, **7**, 2906 and 2914) showed that rhodopsin protein can be separated by gel filtration chromatography, and that it behaves as a globular protein of molecular weight about 28,000 and diameter 46 Å. The retinal molecule has a molecular weight of only one-hundredth of that of the protein, but since it is about 15 Å long, if it is visualised as fitting into a binding site on the surface of the protein, it will span a considerable arc of the circumference. In fact, Saari claims to have removed many of the hydrophilic groups of the protein, which apparently lie on the surface, without disturbing the chromophoric group, and so the binding site may well be a cleft in the interior of the protein. In either case, the chromophoric group will lie in close proximity to a large number of the amino acid residues of the protein—close enough for electrostatic or dispersive forces to act between them.

WHETHER the study of the orientation of ancient monuments ranks as a science or a pseudo-science remains a matter for debate, but there can be no doubt that the topic is once again attracting a great deal of attention. Having formed a focus of interest in the latter years of the nineteenth century and early in the twentieth century, studies of orientation went into decline in the inter-war years: partly, perhaps, because of factors such as excessive emphasis on allegedly significant orientations in Nazi Germany, and partly because the different groups of people involved—mainly astronomers and archaeologists—had failed to agree on the reality and significance of the results.

The recent revival of interest in orientation studies has arisen mainly from new detailed surveys of megalithic sites in the UK, especially those carried out by professor Thom since his retirement from Oxford. He has used these measurements in two ways—to identify the astronomical events indicated by the orientations and to consider the actual layout and scale of the monuments. The consensus of opinion at a recent joint meeting of the Royal Society and the British Academy on ancient astronomy tended to be that the deductions concerning astronomical orientation had firmer bases than those relating to the existence of a standard measure of length in megalithic times. Certainly the former have excited the more discussion. For, unlike most archaeological discoveries which illuminate primarily material culture, they could give a direct, if partial, insight into the level of intellectual sophistication that had then been reached.

Astronomers have in general contributed to the recent growth of interest in studies of alignments more by theoretical speculation than by on-site surveying. Indeed, much of the controversy engendered by the claimed alignments has hinged on the alleged extravagances of some of these

Ancient observatories

from A. J. Meadows

speculations (especially regarding the astronomical implications of Stonehenge). The crucial point behind both the speculations and the arguments hinges on the significance of any measured orientation. After all, astronomical objects have to rise and set somewhere on the horizon: could not the observed alignments be purely coincidental? How convincing an answer can be given to this question depends critically on the accuracy and uniqueness of the data collected.

A group from the Cambridge University Astronomical Society has now re-surveyed with increased accuracy three sites previously described by Thom (this issue of *Nature*, page 431). The most interesting of the three sites is that at Ballochroy in Argyllshire (which Thom has described as one of the most important he knows for observing the solstitial Sun). The new measurements not only support Thom's claim, they also produce a set of dates for the various orientations present that agree remarkably well among themselves, and convincingly suggest that the stones were erected for use around the date 1600 BC. But the other two sites surveyed—at Loch Seil and Loch Nell, both also in Argyllshire—provide much less satisfactory evidence. At Loch Seil, the suggested alignments seem to be non-existent, and at Loch Nell, although the alignments are clearly defined, they do not point to any obviously significant astronomical event.

The sceptic might reasonably comment that one interpretable site out of three suggests that chance may well play a part in producing apparently significant orientations. But chance

would be unlikely to produce orientations that agree with respect to dating, as do those found at Ballochroy. Besides, disturbances must certainly have occurred at some sites during the past four millennia, disrupting alignments that were originally there, and making interpretations ambiguous. The interesting discrepancies are rather those, as at Loch Nell, where the intended alignment seems obvious, but the astronomical explanation does not; for this raises the question whether all, or even the majority of, megalithic monuments were really intended to serve as astronomical observatories. One comment is worth making on this: there is still a need to consider all astronomical bodies that might have attracted attention in megalithic times. So far, attention has concentrated mainly on the Sun, Moon and a few bright stars. However, the planets, especially Venus, were surely equally important in ancient astronomy. The problem, in terms of orientation studies, lies in distinguishing between solar and planetary positions on the horizon.

If we compare studies carried out in recent years with those occurring earlier in the century, the only apparent difference in practical terms is an increase in the accuracy and extent of the site surveys. Why then has the subject re-erupted into the news? I would guess that one reason is a new pervasive interest in the influence of astronomical (or astrological) ideas on the thinking of early man. This is reflected in recent discussions of the astronomical implications of myth, noticeably the detailed analysis in *Hamlet's Mill* by G. de Santillana and H. von Dechend. It appears, too, in the debate over very early archaeological material that, according to A. Marshack, indicates a primitive lunar notation. All these topics are controversial, but, in combination, they suggest the intriguing conclusion that man was numerate long before he was literate.

1973) have suggested that this is due to the stereospecificity of the binding site, which can select one enantiomer from a solution of 11-*cis*-retinal, and thus confer optical activity on the visible absorption. On the other hand, Johnston and Zand (*Biochemistry*, **12**, 4637; 1973) have shown that model aldimines of all-*trans*-retinal with some optically active amines show circular dichroism, so long as the amine has an aromatic group that can couple with the long-wavelength absorption band of the retinal. Thus the fact that rhodopsin shows circular dichroism in

the visible region implies only that the chromophoric group is bound to an optically active amino acid, and that there are aromatic amino acids near the binding site: the retinal is not necessarily in a dissymmetric conformation.

Intact retina

Another physical method of great potential in probing the structures of coloured molecules in biological milieux is the resonance-enhanced Raman effect. In 1970, Rimai, Kilponen and Gill (*Biochem. biophys. Res. Commun.*, **41**, 492; 1970) showed that using this

technique it is possible to study the chromophoric groups of rhodopsin molecules in the intact retina, and thus provided the first direct evidence that the retinal is bound in the form of an aldimine. The intense 488 nm exciting light used in these experiments bleached the rhodopsin, but it has since been shown that use of a 583 nm dye laser permits the recording of the Raman spectrum without undue bleaching, and so it could be established that the chromophoric group is probably in the 11-*cis* conformation (Lewis, Fager and Abrahamson, *J. Raman Spect.*, **1**, 465;

1973). Unexpected bands in the 550–950 cm^{-1} region are ascribed to interactions of aromatic amino acids with the chromophoric group—perhaps these are the same interactions that determine the absorption maximum of the pigment. Confirmation that the aldimine linkage of rhodopsin *in situ* in photoreceptor membranes is protonated has recently been presented by Oseroff and Callender (*Biochemistry*, **13**, 4243; 1974), who also studied the effect of light on the pigment at low temperatures. Illumination gives an equilibrium mixture of rhodopsin and two products, isorhodopsin and bathorhodopsin, which are all interconvertible by light. The conversion of rhodopsin to isorhodopsin requires isomerisation of the chromophoric group from the 11-*cis* to the 9-*cis* form, and the conversion to bathorhodopsin was previously thought to involve isomerisation to the all-*trans* form. However, Oseroff and Callender conclude that the retinal in bathorhodopsin is not in any conformation known from studies of solutions. Bathorhodopsin is the first of a series of intermediates seen in the bleaching of rhodopsin at normal temperatures: the absorption maxima of these lie between 543 and 380 nm, and it is tempting to think that these represent stages in the twisting of the chromophoric group as it progressively breaks free of the binding site. Resonance Raman spectroscopy promises to be a splendid tool in the study of these intermediates, and may also explain the mechanism that determines the absorption maxima of the rhodopsins from different species.

Another component of the rhodopsin molecule that is in the news is the carbohydrate moiety, originally identified by Heller and Lawrence (*Biochemistry*, **9**, 864; 1970) who suggested that it acts as a marker on the surface of the molecule to ensure its correct orientation on assembly into the photoreceptor membrane. Renthal, Steinemann and Stryer (*Expl Eye Res.*, **17**, 511; 1973) have since established that it is not involved in the binding site, for chemical modification of the carbohydrate does not prevent the regeneration of bleached pigment. The group also provides a convenient point of attachment for a 'fluorescent probe' molecule, and a study of energy transfer between the probe and the chromophoric group suggests that these are separated by between 51 and 65 Å—more than the apparent diameter of the protein. This may mean that the rhodopsin molecule is ellipsoidal, or could be explained by the recent studies of Romhanyi and Molnar (*Nature*, **249**, 486; 1974) who have shown by light-microscope histological methods that the saccharide chains of the rhodopsin molecules in fixed frog receptors point

outwards from the molecule. It is suggested that these extend into a hydrophilic region in the receptor membrane, and serve not only to anchor the molecule in a fixed orientation but also to act as a pivot for its rotation. Surprisingly, Saari reports that proteolysis does not remove the carbohydrate moiety, even though this degradation is thought to involve the hydrophilic parts of the molecule most remote from the chromophoric group.

We are in an exciting era of visual pigment research, when many of the old dogmas are being re-examined and new physical techniques now permit measurements to be made on the behaviour of rhodopsin in intact photoreceptor cells. Perhaps we shall soon have a real insight into the structure—and finally the function—of this strange molecule.

Basin tectonics

from A. Hallam

It is no discredit to the elegant simplicity of plate tectonics that only in the oceanic sector does it throw significant new light on vertical (as opposed to lateral) motions of the Earth's surface. Plate tectonics revolutionised the study of structural evolution by accounting for major tectonic features of the Earth by comparatively few lateral motions and interactions of lithospheric plates. Not surprisingly, the success of the new concept pushed into the background related problems which have been wrestled with since the time of Lyell and Darwin—problems associated with the uplift of continental mountain ranges and plateaux and the subsidence of basins or continental margins. In the preparation of dishes for the connoisseurs of world tectonics we seem to have become obsessed with plates and neglected basins.

The widespread indications of marginal downwarping of continents such as seaward descent of erosion surfaces and warping of marine terraces, seaward dip and thickening of Cretaceous and Tertiary sediments, palaeogeographical evidence of founded offshore land areas and, more controversially, submarine canyons, led many geologists to agree with Kuenen's judgement of a quarter century ago that it was "difficult to avoid the conclusion that marginal areas have subsided to form part of the present deep sea floor". Now isostatic considerations demand that this could only happen if the continental crust were substantially thinned or in some mysterious way converted into denser oceanic-type crust. The Soviet tectonician Belousov has gone so far as to invoke extensive 'oceanisation' of continental crust to

account for the ocean basins; he has in consequence totally rejected plate tectonics and explains first order structures exclusively by vertical motions. One need not go so far as to adopt this extreme heretical position to accept that something strange and as yet inadequately explained has been going on at continental margins.

Subsidence of parts of the North Atlantic continental margins has recently been shown by geophysical work and deep sea drilling to be even more extensive than recognised hitherto. Thus Talwani and Eldholm (*Bull. Geol. Soc. Am.*, **83**, 3575; 1972) have demonstrated that the Vøring Plateau off Norway, occupying an average depth of 2,000 m, is subsided continental rather than oceanic crust, with an eastward-descending ridge of probably Precambrian basement buried beneath a considerable thickness of Palaeozoic and younger sediments; it can thus be compared with, for instance, the Rockall Bank. East of Newfoundland is another area of deep sea floor which gives indications of being subsided continent (Ruffman and van Hinte, *Geol. Surv. Can. Paper* 71–23, 407; 1973). Drilling on the Orphan Knoll seamount has located Middle Jurassic continental coal measure deposits, which points indubitably to over 2,000 m of subsidence since that time. Ruffman and van Hinte argue for a much more extensive subsidence, of as much as 3,500 m, of a vast area of deep sea floor to the south and west of Orphan Knoll.

This work has some interesting implications. It suggests for instance that the so-called Quiet Magnetic Zone is not, as most geophysicists have assumed, true oceanic crust produced by spreading at a time of no magnetic reversals, but subsided continental margin, as I inferred a few years ago (*J. Geol.*, **79**, 129; 1971). This would explain why the position of the Quiet Magnetic Zone is always near the continental margin irrespective of the postulated age of oceanic opening. Second, the common acceptance of the 1,000 m isobath as the effective edge of the continents for the purpose of least squares fits, to obtain their pre-drift positions, is seen to be inaccurate and leads to their being placed too close together. This could well account for the most awkward feature of the celebrated Bullard fit of the Atlantic continents, namely the lack of space for Central America. In terms of plate theory the marginal downwarping can be explained as follows. Prior to or contemporary with initial oceanic rifting the continental crust was bowed up by expanding hot mantle and thinned slightly by erosion but more substantially by tensional stretching accommodated by normal faulting. As the continents subsequently moved apart

Hexokinase in human muscular dystrophy

from a Correspondent

CONTROVERSY abounds at the moment about the pathogenesis of the severe X-linked form of muscular dystrophy, which is known as the Duchenne type and which usually results in the affected boys being unable to walk by the age of about 10 years and rarely surviving beyond the age of 20. Within recent years McComas, Sica and Currie (*Nature*, **226**, 1263; 1970) have suggested that the progressive degeneration of skeletal muscle which occurs in this condition may be the result of a primary defect in the motor neurones, whereas Hathaway, Engel and Zellweger (*Archs Neurol.*, *Chicago*, **22**, 365; 1970) have postulated a process of progressive ischaemia of muscle because of a superficial similarity of the pathological changes in skeletal muscle in such cases to those produced by experimental muscle embolism in animals. These hypotheses have challenged the traditional view that this and other forms of genetically determined muscular dystrophy are likely to be due to a primary biochemical abnormality of the muscle cell itself, though it is certainly possible that a genetically determined biochemical defect could influence the function of both motor neurones and skeletal muscle.

Ellis, Strickland and Eccleston (*Clin. Sci.*, **44**, 321; 1973) have previously found that muscle obtained by biopsy from patients with Duchenne type muscular dystrophy is less able than is normal muscle to utilise glucose by the usual pathway of glycolysis. They found that in such tissue there was partial conversion of glucose to fructose instead of to glucose 6-phosphate under certain metabolic conditions. The

conversion of glucose to glucose 6-phosphate is normally catalysed by hexokinase, an enzyme present in mammalian tissue in the form of several isoenzymes which differ in their K_m values for glucose.

Strickland and Ellis have now found certain abnormalities of hexokinase isoenzyme mobility when studied electrophoretically in muscle obtained from patients suffering from X-linked Duchenne type muscular dystrophy and the similar but more benign X-linked Becker variety (this issue of *Nature*, page 464). They find that tissue obtained from patients with these two diseases shows three, instead of the normal two, bands for hexokinase, two of which move relatively faster than do the bands of the normal isoenzyme II, and an additional slower-moving band close to isoenzyme I. Similar isoenzyme changes were found in liver, brain and sciatic nerve obtained at post-mortem from patients with Duchenne type muscular dystrophy. As the authors point out, this alteration in hexokinase isoenzyme II in a number of tissues obtained from patients with muscular dystrophy might account for a restricted flow of glucose through the hexokinase step in tissues where isoenzyme II is required. If such an abnormality were to prove to be of fundamental importance in the pathogenesis of muscular dystrophy, it could certainly account for abnormalities in the function of both neurones and muscle cells in this disease. Confirmation of this work and further studies of hexokinase isoenzyme behaviour in muscle and other tissues obtained from patients with X-linked muscular dystrophy will be awaited with interest.

the trailing margins continued to subside as the newly created ocean floor cooled progressively and hence became more dense. It remains to be seen whether this model can account in a satisfactory quantitative way for several thousand metres of subsidence since the early Cretaceous, apparently regardless of the age of opening.

Although the Atlantic margins offer interesting problems their solution seems relatively straightforward compared with those posed by substantially land-locked marine basins such as the Gulf of Mexico, the Black Sea and parts of the Mediterranean Sea. For the Black Sea Brinkmann (*Am. Ass. Petrol. Geol. Mem.*, **20**, 63; 1974) has

outlined the abundant palaeogeographical evidence that it was the site of a terrestrial sediment source for much of the Palaeozoic and early Mesozoic. Yet seismic surveys reveal that the crust underlying the Black Sea has suboceanic thicknesses. Probably the most plausible interpretation of the existing data is that thick Upper Cretaceous and Tertiary sediments rest on a much attenuated continental crust. The obvious question arises, what has caused the thinning? Unlike the case of the Atlantic margins it cannot readily be related to a generally accepted plate tectonic model.

The case of the Mediterranean basins is perhaps even more intriguing, since

we have two quite different interpretations of their origin, one invoking lateral, the other vertical movements. Western Mediterranean basins such as the Balearic Basin and Tyrrhenian Sea have a crust which is at least suboceanic if not fully oceanic in character, though no magnetic anomalies indicative of seafloor spreading have been discovered. Palaeomagnetic evidence for the anticlockwise rotation of Corsica, Sardinia and Italy has been held to confirm Carey's proposal that the western Mediterranean basins are sphenochasms, that is, wedge-shaped sectors of ocean floor created by the movement apart of slabs of continent. Hsü and others have adopted this model to account for the existence in the deep basins of substantial deposits of late Miocene NaCl and CaSO₄ salts which bear the characteristics of continental salina and sabhka deposits. To explain this apparent paradox a boldly imaginative hypothesis has been put forward (Hsü, *Nature*, **233**, 44; 1971; Hsü *et al.*, *Init. Rep. Deep Sea Drilling Project*, vol. 13, 1203; 1972). After Africa closed on Europe during the early Tertiary and sphenochasms opened up in the intermediate zone, a stage was reached when a series of land-locked basins several thousand metres deep were created in the Miocene. Because the climate was warm and arid the Mediterranean remained substantially dry, except for shallow brine pools in the deep basins, where salts began to precipitate. River canyons were cut down as much as 2,000 m through the surrounding continental margins to a base level well below ocean level. Subsequently, in the Pliocene, the Straits of Gibraltar were opened up and sea water flooded in from the Atlantic, rapidly drowning the basins and canyons and allowing normal deep sea muds to be deposited. As a defence against an admittedly extravagant, though attractive, hypothesis, Hsü has quoted Sherlock Holmes: "When you have excluded the impossible, whatever remains, however improbable, must be the truth."

But can the 'impossible' be excluded? Let us consider some other facts. There is an old idea that the Tyrrhenian Sea, like the Black Sea, is the site of a subsided landmass. This idea has received impressive support from the dredging of continental metamorphic rocks from the margin of a tilted fault block in the centre of the sea, implying late Tertiary subsidence in excess of 3,000 m (Heezen *et al.*, *Nature*, **229**, 327; 1971). The occurrence of a parallel series of ridges and troughs suggests a series of fault blocks like the Great Basin regime of the western United States and implies considerable thinning of continental crust by tension. More recently, combined geophysical

and stratigraphical work in the Balearic Basin and the margins of Catalonia has provided strong support for substantial subsidence in the recent geological past (Stanley *et al.*, *Geology*, 345, July 1974). Prominent stratigraphical horizons of Miocene or younger age have been demonstrated to be downwarped or down-faulted towards the basin floor by as much as 2,000 m. The submarine canyons evidently result from the drowning of previously emergent land margins to depths of more than 1,000 m. In other words the Miocene salt was originally deposited at or slightly above sea level and has subsequently subsided with the underlying crust.

One of these explanations must be wrong. If we are to adhere to Hsü's hypothesis how are we to account for the apparently good evidence of basinal subsidence? If accordingly we reject it we must presumably also reject the palaeomagnetic evidence for the rotation of Corsica, Sardinia and Italy and must also try to explain up to 3,000 m subsidence in the last 15 million years, and the substantial loss of continental crust. Is there any correlation between the basin subsidence and the uplift of certain circum-Mediterranean mountain areas? To give just one instance, locally in Tunisia there has been at least 3,000 m uplift since the late Miocene. If the crust has been thinned by tension this must presumably have operated radially, as also would be the case in the Gulf of Mexico, which similarly has oceanic crustal structure, buried salt (probably Jurassic) in its deepest part and suggestions from palaeogeography of occupying the site of an ancient landmass. To what extent are the pronounced changes in elevation a consequence of changes in crustal thickness or density, or variations in density of the upper mantle? Can the underlying phenomena be readily reconciled with our present geophysical concepts, which lean heavily on plate tectonics, or are other mysterious forces at work?

My dear Watson, it's not so elementary!

Oceanic ridges as collapse calderas

from Peter J. Smith

THERE are obvious differences, both in structure and behaviour, between the long linear oceanic ridge and the geographically localised volcano. But there are also significant similarities, for in their active forms both features are associated with very young volcanic rocks, rocks which have undergone high temperature metamorphism and high heat flow. Moreover, some volcanoes produce lavas similar to the basalts found on the ocean floor. These



A hundred years ago

THE words of Mr Disraeli on Monday night with regard to University Reform are also very cheering to those who wish to see some decided action taken towards the thorough reform of our Universities. Mr Disraeli's words were very strong, so strong indeed as to amount to an assurance that Government really means to take into serious consideration this session the Report of the University Commission. "It is our opinion," the Prime Minister said, "that no Government can exist which for a moment maintains that the consideration of University Reform, and consequently legislation of some kind, will not form part of its duty." These words give out no uncertain sound. Mr Disraeli said, moreover, that when the Report was presented at the end of last session, the Colleges were not assembled. It would be interesting to know whether the Colleges have yet met to consider the Report, and whether they are likely to act on this hint of the Premier and take some internal action—commence the work of reform from within, instead of waiting until they are driven to it by forces from without.

from *Nature*, 11, 292; February 11, 1875

characteristics suggest that ridge axes may be underlain by magma chambers at a depth of a few kilometres. Is it possible, then, that the study of volcanoes with magma chambers will help in the understanding of the less accessible ridges?

In the hope that such a comparison would be illuminating, Francis (*Geophys. J.*, 39, 301; 1974) has analysed the activity of the Fernandina Caldera (Galapagos Islands), whose floor collapsed in 1968. In the early part of that year, Isla Fernandina, a large basaltic shield volcano, had an elliptical caldera with a flat floor about 7 km² in area. But on June 11 the caldera floor began to subside in a series of short bursts and along a steep pre-existing elliptical boundary fault. According to Simkin and Howard (*Science*, 169, 429; 1970), the increase in caldera volume caused by the collapse was 1–2 km³, and the lowering of the floor (unevenly) by an average of 150 m represented a potential energy loss of 10²³–10²⁴ erg.

The seismic energy released was several orders of magnitude lower than this (10¹⁹–10²¹ erg), but its effect was hardly less spectacular. Whereas in 1967 and 1969 earthquakes were reported from the area at an average rate of about one every two months, at

least 599 shocks occurred during the period 12–23 June, 1968. The largest of these events, most of which were shallow, had maximum magnitudes $m_b=5.4$ and $M_s=5.2$. But more significantly, the magnitude–frequency plot, whether using m_b or M_s , is best fitted by two straight lines rather than one; there is a definite change in slope (b value) at about $m_b=4.9$ or $M_s=4.5$.

The two distinct b values for M_s data were first recognised by Filson *et al.* (*J. Geophys. Res.*, 78, 8591; 1973), who were, however, unable to explain them. But Francis has now been able to extend the Filson model to give a straightforward explanation of the seismic data. The caldera floor is regarded as the top of a plug capping a magma chamber (plug and chamber being cylindrical in the simplest form of the model). The fact that the Fernandina caldera collapsed along a pre-existing fault surface implies that under normal circumstances the plug is not supported structurally but by the pressure of the magma beneath. A collapse will occur when, for one reason or another, the magma chamber pressure is reduced to the point at which the shear stress on the fault surface exceeds the fault strength. When this happens, the plug will fall to a new equilibrium position.

From the model, and using a modified form of Press's (1967) formula relating fault length to earthquake magnitude, Francis shows that the maximum magnitude of shocks produced along the main caldera fault should be about 5.4 (m_b). This agrees with observation. But a model involving a falling rigid plug is not entirely satisfactory insofar as, in the real Fernandina collapse, further faulting occurred at the north-west end of the caldera. Here there was no appreciable subsidence; and flexing of the caldera floor produced a band of fractures aligned normal to the caldera's long axis. The length of this fracture zone is an order of magnitude smaller than that of the main fault and the predicted maximum magnitude of shocks from the zone is correspondingly low at $M_s\sim 4.0$.

Further calculation shows that the larger shocks produced at the main fault are associated with low average stresses and small stress drops—which implies high b values. By contrast, the tensional fracturing in the north-west has stresses comparable to the average stresses for most earthquakes; the b values are thus also comparable (normal). In other words, the two distinct b values observed for the Fernandina collapse swarm arise from two different types of earthquake.

So the seismic characteristics of the Fernandina caldera collapse are broadly explicable in terms of observed physical processes. But how does this relate to oceanic ridges? The point that

Francis makes is that in many respects the seismicity of slow-spreading ridges (fast spreading ridges such as the East Pacific rise are rather different) is similar to that of the caldera collapse episode. At the mid-Atlantic ridge, for example, all earthquakes are shallow and none is very large (maximum $m_b \sim 5.6$ in rift zone), rift zone earthquakes seem to be confined to the median valley, the b value of rift zone events is usually significantly higher than for transform fault events, rift zone events exhibit normal faulting, and earthquakes swarms are common along rift zones. Moreover, earthquake swarms associated with spreading axes in the Gulf of California have been shown to be low stress events.

Francis thus proposes that rift zone seismicity along slow-spreading ridges is generated by a series of caldera collapses. The median valley floor of the mid-Atlantic ridge, for example, is constantly collapsing at various points, the locations of which will be governed by the configuration of the magma chambers and of the overlying topography. Generally, collapse will occur along pre-existing faults, although when such faults have moved outside the influence of the magma chamber (by lithospheric spreading) new faults will form along the chamber's lateral limits. The width of the median valley floor thus roughly defines the maximum width of the magma chamber.

Where is the myosin?

from Dennis Bray

THE history of muscle research would have been very different if striated muscle had not been built in such a well ordered fashion. The almost crystalline myofibrils, composed of parallel filaments in segmented arrays, first caught the eye of light microscopists and later provided material for electron microscopy and X-ray diffraction. The information they provided on the positions and mode of interaction of the two kinds of elemental filament was unique and independent of biochemical analyses of their component proteins, such as actin or myosin.

Curiously, we are now in precisely the opposite position with non-muscle cells. Most eukaryotic cells have been shown to contain proteins that are chemically very similar to actin and myosin and which probably work in the same way, at the molecular level, as their counterparts in muscle. But, with the exception of the bundles of actin-like filaments that some cells possess close to their membrane surfaces, we have little idea where these proteins are within the cell. How they might be linked to other cellular constituents, or produce coordinated movements, are still open questions.

Some of the difficulties in placing these proteins within the cell are illustrated by the variety of results recently reported for myosin. The myosin of blood platelets and cultured fibroblasts seems to be more soluble than muscle myosin, and does not condense so readily into thick filaments at physiological ionic strength. If these cells are disrupted and subcellular fractions prepared by differential centrifugation (in the now time-honoured way) then most of the myosin is found in a soluble, non-sedimentable form (Ostlund, Pastan and Adelstein; *J. biol. Chem.*, **249**, 3903; 1974). To judge from this direct and quantitative method most of the myosin is freely diffusing within the cell, either as single molecules or as small aggregates.

Yet if a different technique is used a different answer is obtained. The recent work of Weber and Gröschel-Stewart (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 4561; 1974), in which cultured fibroblasts are fixed and exposed to anti-serum to uterine myosin, shows that at least part of this protein is to be found in filaments. The striking pictures they obtained by immunofluorescence show long straight strands extending across the cell, often in parallel alignment, and with a periodicity in the staining revealed at higher magnification. Since there is some reason to think that these strands may also contain actin an arrangement similar to that in myofibrils may be present, and it is tempting to think that such strands may be able to contract.

And the possibilities do not end there. Again using immunofluorescent techniques but this time applying them to intact cells, two laboratories have reported the presence of myosin on the outer surfaces of cells (Gwynn *et al.*, *J. Cell Sci.*, **15**, 279; 1974; Willingham *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4144; 1974). Conscious, perhaps, of the unexpected nature of their findings these workers have carried out extensive controls. Willingham and co-workers in particular, compared antisera to three types of myosin: fibroblast, smooth muscle and striated muscle, and found that only the first of these gave the surface reaction. Among other tests, they carried out the important one of absorbing anti-serum to the cells and showing that upon elution it still cross reacted with myosin.

It is true that immunological evidence in the absence of direct biochemical tests is hard to defend against all objections. Myosin, in particular, is a difficult protein to purify and few would claim that their preparation could not contain up to 5% of other components, perhaps from the surface. In addition, the possibility of adventitious contamination by myosin from

lysed cells is always present. Nevertheless, an interaction of contractile proteins with cellular membranes is now widely expected and could have as a consequence the exposure of part of the myosin molecule to the outside of the cell. It will be most exciting if this is true, and worth the search for independent methods of testing.

The present picture seems confusing. We have evidence that myosin is free within the cytoplasm, present in strand-like filaments, and closely associated with the cellular membrane. But these are not necessarily incompatible results. Only one of the methods used to detect myosin is quantitative—that of measuring its ATPase activity—and the immunological results could apply to only a small fraction of the myosin within the cell. It is entirely conceivable that this protein could exist in more than one state of aggregation or be associated with more than one subcellular structure. If so, then the factors that control its distribution will necessarily be critical for the movements of the cell.

Proline implicated in halophyte osmotic adjustment

from Peter D. Moore

JUST as adaptations to cold and to heat seem to go hand in hand (*Nature*, **253**, 11; 1975), so do adaptations in many plants to drought and salinity. Water-stressed plants of several species (for example broad bean: Stewart *et al.*, *Plant Physiol.*, **41**, 1585; 1966, and barley: Singh *et al.*, *Nature new Biol.*, **236**, 188; 1972) have shown an accumulation of the free amino acid proline. Singh *et al.* showed that different varieties of barley had different capacities for the accumulation of proline in response to water stress.

Waldren and Teare (*Plant and Soil*, **40**, 689; 1974) have now examined proline levels in intact plants of sorghum and soybean and at the same time measured stomatal diffusive resistance and leaf water potential as indications of water stress. In soybean, proline only began to accumulate when the stomatal diffusive resistance exceeded 37 s cm^{-1} and when a leaf water potential of -14 bar was reached. By this time the plants showed loss of turgor. In the case of sorghum, proline accumulation occurred only when stomata were completely closed and when the leaf water potential had reached -24 bar. Again, incipient wilt was evident at this stage, so proline concentrations cannot be regarded as a sensitive indication of water stress for these species.

This type of association of free proline with drought response in plants

has led Stewart and Lee (*Planta*, **120**, 279; 1974) to investigate the possibility that proline may be associated with the tolerance of physiological drought in halophytes. They analysed the free amino acid content of a number of halophytes from salt marshes in western Britain and found that in many (for example *Aster tripolium*, *Armeria maritima*, *Puccinellia maritima* and *Triglochin maritima*) the concentration of proline was higher than that of any other single amino acid, often by a factor of ten. In some species proline represented 70% of the free amino acids and in most it exceeded 30%. But *Plantago maritima* had a low proline content; this exception demonstrates that proline accumulation is not a universal feature of halophytes.

Using *Triglochin maritima* Stewart and Lee were able to show that proline build-up is enhanced by high salinity in the surroundings, just as Waldren and Teare had demonstrated in the case of water stress. They also found that different populations of certain species, which were geographically isolated and which had experienced different salinities over long periods of time, had varying capacities for the accumulation of proline. For example, *Armeria maritima*, the thrift, is a species which occurs both as a maritime halophyte and as a species of arctic-alpine habitats on many British mountains. Palaeoecological studies suggest that the species was widespread in distribution during the cold and disturbed conditions at the close of the last glaciation, some 10,000 years ago. Populations have subsequently been isolated on mountain tops and coastal habitats as forest has spread and eliminated this shade-intolerant species from many habitats. When tested under a variety of salinities, *Armeria* populations from inland situations showed only a limited capacity for proline accumulation. The response of coastal *Armeria* samples was about three times as great as those from inland. Such a difference in response is reminiscent of the varietal adaptation to proline accumulation as a response to drought found in barley (Singh *et al.*; 1972).

It is known that the enzymes of some halophytes are not able to operate in media of high salt concentration (for example, the work of Flowers, *J. exp. Bot.*, **23**, 310; 1972, on *Sueda maritima*). Sodium chloride within halophytes must, therefore, be physically separated from the cellular enzyme systems, presumably by storage in the vacuole. Stewart and Lee have examined the effect of elevated proline levels upon the activity of several enzymes in *Triglochin maritima*; they found no depression of activity. They suggest, therefore, that proline could provide the means of cytoplasmic osmotic

adjustment in those halophytes where proline accumulation has been observed. Perhaps it serves a similar function in drought-stressed tissues where osmotic compensation may be required within the cytoplasm of affected cells. Apart from the elucidation of the precise role of proline in these processes, one of the most interesting developments in this work is likely to emerge from the exceptions to the general rules—exceptions like *Plantago maritima*.

Laser studies of atomic and molecular collisions

from G. Duxbury

In the last fifteen years there has been an increasing interest in the experimental and theoretical study of the energy transfer processes occurring in atomic and molecular collisions. One of the main experimental techniques which has been used is that of crossed molecular beams, which requires very expensive ultra-high vacuum apparatus. An alternative way of obtaining the information is based upon experiments using single frequency narrow linewidth lasers. In these experiments use is made of the velocity selective interaction of monochromatic radiation with gaseous atoms and molecules. The information obtained from the laser experiments is similar to that from the crossed beam experiments, but does not require ultra-high vacuum techniques for its success. In addition, information can be gained, not only on the changes of translational energy, but also on changes in rotational energy. As examples of the types of experiment that can be carried out, two different ones are described, one of which has been carried out and one which has recently been proposed.

If a single-frequency laser emits radiation of frequency ν which differs slightly from the resonance frequency ν_0 of the atoms or molecules which absorb the radiation, the Doppler shift will make the laser radiation appear exactly on resonance only for those molecules whose component of velocity along the laser beam is $v = c(\nu - \nu_0)/\nu_0$, where c is the velocity of light. If the molecule irradiated is allowed to collide with a heavy target, the velocity dependence of the collision cross section for the production of a particular excited state can be determined by measuring the ratio of the fluorescence from this state, to that of the laser excited resonance fluorescence as the laser frequency is changed. Phillips and Pritchard (*Phys. Rev. Lett.*, **33**, 1254; 1974) have proposed that this technique should give velocity resolutions comparable with that obtained

using the crossed molecular beam method, providing that the target is at least five to ten times heavier than the atom or molecule excited. They also suggest that since the velocity distribution in the laser experiment is known exactly, the true cross section can be deconvoluted from the measured one very accurately, so that this compensates for the slightly poorer velocity resolution of the laser experiment. It is proposed that, in some circumstances, state selection can be achieved by the use of a polarised laser beam. This should give a preferential orientation of either the orbital angular momentum vector, or of an electric dipole moment, along the direction of propagation of the radiation. The experiments described above are for the linear absorption region.

An alternative method of studying energy transfer that has already been demonstrated utilises saturation effects produced in low pressure gases by high power lasers. Under these conditions 'holes' are produced in the population of energy levels associated with the absorbing transition. Good examples of this type of behaviour are provided by the elegant double resonance experiments carried out by Freund, Johns, McKellar and Oka (*J. chem. Phys.*, **59**, 3445; 1973). They studied two, three and four level systems in the vibration rotation spectrum of ammonia. The main effects that they demonstrated were the monitoring of a hole in the population of a vibration-rotation energy level caused by a strong two-photon pumping source, by a weak two-photon signal source, and the observation that holes burnt in the population of one level could be transferred to other levels by collisions. Two-photon pumping is not an essential requisite, and can be replaced by tunable laser sources once these become available. By fine tuning both the pumping and the monitoring sources different velocity molecules were studied, and by larger variations of the monitoring source energy transfer to different rotational energy levels was studied. Thus from one type of experiment it is possible to obtain information about both the magnitude of the intermolecular interaction $V(r)$ from the translational changes, and the angular dependence of the intermolecular interaction $V(\theta, \phi)$ from the rotational changes.

These sorts of study are only possible when the predominant cause of spectral line broadening is the Doppler effect, as in the infrared and visible regions. In the microwave region the lines are predominantly pressure broadened and double resonance then gives information only on rotational state changes, but not on changes in translational energy.

review article

Recent advances in electrical recording from the human brain

D. Regan*

New techniques for recording evoked potentials to sensory stimulation have led to applications with both theoretical and practical implications. Evoked potentials can be used as a diagnostic tool in some pathological conditions, as well as shedding light on the mechanisms by which sensory information is processed in the brain.

A FLOW of visual information to the eyes, auditory information to the ears or tactile information to the skin is represented by nervous discharges whose information content is successively reorganised as the signals pass from neurone to neurone on their way to visual, auditory or somatosensory areas of brain cortex where yet further processing takes place. The weak electrical currents generated here pass through the skull and can be recorded in the form of voltages, known as evoked potentials (EPs), between electrodes held on to the scalp. Although evoked potentials are generated mainly in the cortex, they can be used as tools to study the ways in which the brain sequentially processes sensory information as it ascends from peripheral to successively more central sites through the nervous system. They can also be used to reveal parallel processing in the brain, in which different types of information are handled simultaneously in separate channels.

During the past decade, descriptions of the ways in which single neurones in animal brains respond when the sensory end organ is stimulated have become a major influence in originating models of sensory information processing, and in this way have prompted a great many psychophysical studies designed to test the extrapolation of single-neurone studies to human perception. It is a long way from the electrical activities of nerve cells in cat or monkey brain to human conscious perception, and EP recording offers a promising means of bridging this interspecies gap. A second, quite different strength of EP recording is that it offers access to certain activities of both the normal and diseased brain that do not intrude into consciousness and so cannot be directly studied by psychophysics or subjective sensory tests. A third area in which EPs offer unique possibilities is in the objective testing of sensory function, especially in young children.

It is rare for a single sensory stimulus (for example one clap of the hands) to produce an EP so large and free from noise that it can clearly be seen in a normally amplified recording from scalp electrodes. More commonly the electrical activity of the rest of the brain is so prominent that the EP is buried in background noise. The most widely used method of enhancing the signal-to-noise ratio is signal averaging^{1,2}. An abrupt stimulus (for example a click) is repeated many (n) times, separated by intervals that are sufficiently long to allow the brain to settle down to its

undisturbed state between repetitions, and the n responses are electronically summed. If the EP to each stimulus is identical and occurs at exactly the same time after stimulation, the summed EP will be n times larger than a single EP, but the background noise will sum more slowly since it has no fixed relationship to the stimulus. This is called a transient EP because it is the transient response to abrupt stimulus such as, for example, a brief flash of light, the

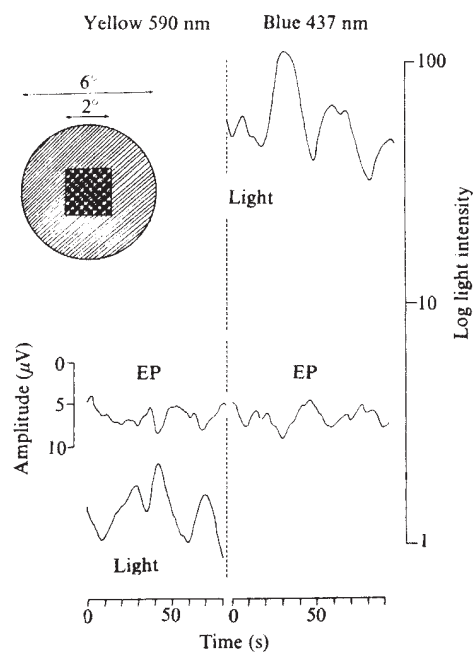


Fig. 1 Evoked potential feedback when brain signals control the stimulus. The visual stimulus shown in the insert was a red pattern of bright and dark checks that exchanged places six times per second. Superposed on the pattern was a desensitising patch of light. The pattern elicited a 6-Hz evoked potential, whose amplitude continuously controlled the intensity of the desensitising light by means of a neutral density wedge in the desensitising beam. This feedback was set to maintain evoked potential amplitude constant at 6 μ V. When the colour of the desensitising light was changed from yellow to blue, the evoked potential immediately moved the wedge so as to increase the intensity of the desensitising light by about 40 times. Since the effect of the red (676 nm) pattern is restricted almost entirely to the red channel, this means that this channel is about 40 times more sensitive to yellow than to blue light. (Modified from Regan⁸.)

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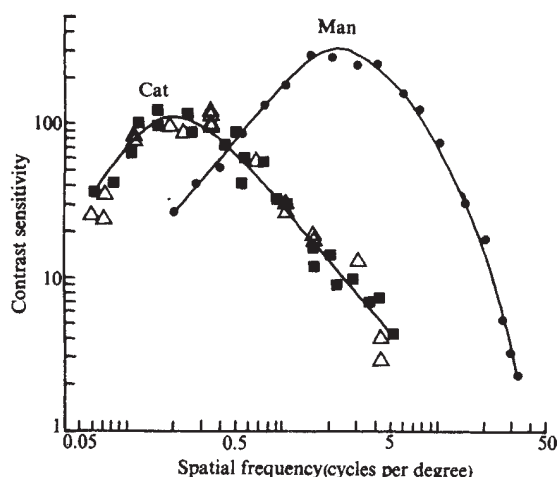


Fig. 2 Pattern vision in cat and man compared by means of evoked potential recording. The filled squares are the "contrast thresholds" determined by evoked potential recording for three cats¹⁴. The open triangles are contrast sensitivities determined behaviourally for two other cats¹⁷, and closely agrees with the evoked potential data. The solid circles are contrast sensitivities measured in man¹⁶, where evoked potential and psychophysical estimates agree also. In comparison with the human curve, the cat's is displaced to lower spatial frequencies by a factor of about ten; a somewhat similar effect would result from viewing the world through reversed binoculars so that everything looked ten times smaller.

onset of steady illumination, or the cessation of a long train of rapidly-repeated clicks.

Steady-state EPs are quite different from transient EPs. When a rapidly repeated stimulus is switched on, there is first a transient response which then gradually settles down to a steady-state EP waveform that repeats at the same frequency as the stimulus (f Hz)³⁻⁵. Here, the stimulus is precisely defined in frequency rather than time so that the steady-state EP can be recognised in the midst of the brain's background noise since it is composed of components (harmonics) whose frequencies are exactly f , $2f$, $3f$ Hz and so on. A Fourier analyser can automatically carry out the required computations, display the amplitude and latency of the steady-state EP, and confirm that the EP fulfils the necessary condition of remaining constant from moment to moment throughout the recording^{3,4}.

In some studies transient and steady-state EPs give complementary insights into brain function. From a practical point of view the steady-state method has the advantage of speed (it is up to 100 times faster)⁷, which may be especially important in clinical studies.

Using Fourier analysis of the waveform, EP amplitude can be displayed as a moment-to-moment running average, thus providing feedback to the experimenter so that he knows at once what result he has produced by changing some stimulus parameter^{3,6}. A different way of utilising this feedback is to arrange that, by electronic means, the EP itself continuously controls the stimulus so as to maintain EP amplitude constant⁸⁻¹⁰. Then the experimental subject's experiences are slightly disturbing: if he consciously tries to blur a stimulus pattern by defocusing his eye, the stimulator opposes him so that the pattern's visibility does not seem to change. The experimenter, however, obtains through this technique a direct measurement of EP sensitivity, instead of an indirect one through amplitude measurements, with the advantages of increased precision and much shorter experiments as, for example, in measuring approximations to the action spectra of the eye's colour mechanisms⁸⁻¹¹ (Fig. 1).

Experimenters searching for the neural basis of conscious perception have looked for relationships between psychophysical measurements of sensation and evoked potentials

in human subjects. In such experiments, evoked potential recording offers a bridge between the detailed subjective reports and quantitative measures of conscious perception that can be obtained from human subjects and the recordings from individual nerve cells in the brains and sensory pathways of animals.

For example, the logarithmic relationship that has been shown to obtain between the amplitude of EPs elicited by a sinewave grating and the objective contrast of the grating^{12,13} corresponds to the logarithmic relationship between stimulus contrast and subjective perception of contrast^{14,15}. The relationship between contrast and EP amplitude has been extended to cats^{16,17} and followed up with studies on the effect of grating contrast on the firing frequencies of single neurones on the cat's visual pathway¹⁸. These experiments showed that the logarithmic relationship held for just one class of neurones, the so-called simple cells of the visual cortex, whose behaviour thus seems to provide a basis for stimulus contrast perception.

The question of whether EP generation fails when the stimulus is made just too weak to produce a sensation has attracted a good deal of attention. There have been encouraging early reports of the use of EPs to auditory stimulation in assessing the bearing of babies and infants¹⁹⁻²³, although some later reports have been less optimistic²⁴. A straightforward way to estimate EP threshold is to graph EP amplitude against stimulus intensity in coordinates that give a straight line plot, and then extrapolate to zero EP amplitude. This method has been used for auditory EPs¹⁹⁻²³, and for visual pattern EPs^{12,13,25,28,29} in both cases giving EP thresholds that agreed well with subjective (psychophysical) thresholds. This latter finding for pattern EPs complements earlier reports that EPs could not be evoked by stimulating an eye with pattern during periods when pattern vision through that eye was suppressed by interocular rivalry³⁰ or by interocular suppression³¹⁻³³.

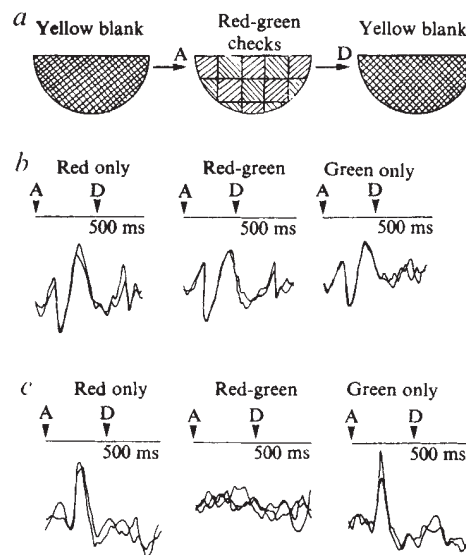


Fig. 3 Evoked potentials produced by a pattern defined only by colour differences. *a*, Stimulus: an unpatterned yellow patch abruptly changed to pattern of equiluminant red and yellow checks (appearance) and then back again (disappearance) without any change in total light flux. The traces marked red-green show that EPs to appearance (A) and disappearance (D) of pattern are clear in normal (*b*) but absent in colour blind (deuteranopic) subjects (*c*). The pattern of red-green checks is made up of a pattern of bright and dark red checks superposed on a pattern of bright and dark green checks. Traces marked red and green show that the constituent monochromatic patterns give clear EPs in both normal and colour blind subjects. (Modified from Regan and Spekreijse⁵².)

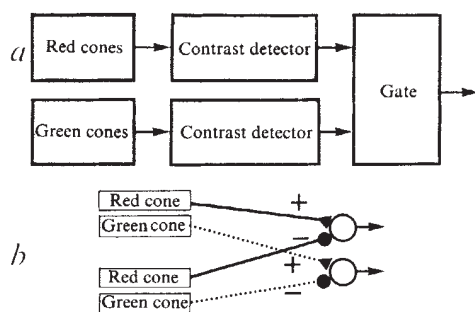


Fig. 4 Pattern information is handled in parallel red and green channels which fuse before the stage of perception. *a*, Red and green images cannot be simultaneously in sharp focus because of the eye's chromatic aberration, but when one or other image is momentarily sharp the corresponding colour channel feeds a strong signal to the gate, the function of which is to transmit preferentially the stronger pattern signal, possibly by means of inhibition between red and green pattern channels. Consequently it is the more sharply focused image that is seen at any given moment independently of whether it is the red or the green image, and furthermore beyond the gate the spectral sensitivity of the pattern channel is effectively photopic rather than being most sensitive to red or to green. *b*, Illustration of one possible arrangement of neuronal connections at the most peripheral stage. Excitatory signals shown by +, inhibitory by -. Uniform illumination by any colour gives zero final output. An output is, however, produced when a bright-dark boundary (or a red-green boundary) is positioned between the cone pairs. Note that this arrangement is by way of illustration only. Many other possible schemes of lateral inhibitory connection would also give sensitivity to spatial contrast borders.

Particularly for pattern stimulation, these findings offer an objective means of estimating psychophysical threshold in animals that may well prove important in future studies of the neural basis of sensation (Fig. 2). For example, such findings promise an alternative to the laborious behavioural methods in animals for detecting the effects on the visual pathway of an impoverished visual environment or artificial rotation of the visual world in early life.

It should not, however, be assumed that EP and psychophysical thresholds always coincide, even roughly. For example, flickering the intensity of an unpatterned patch of light gives EPs that bear no relation to subjective threshold. Whole experiments can easily be carried out with stimuli so weak that the subject never sees them³⁴. More significantly, the same increase of flicker frequency can increase EP amplitude, while abolishing the sensation of flicker³⁵. This dramatic difference between EPs elicited by changing stimulus contrast (pattern) and by changing overall stimulus intensity is consistent with the suggestion that the two types of EP are generated by different populations of cortical cells³⁶.

In practice the two types of EP can easily be confused, since stimulating the eye with a pattern does not necessarily give a pattern EP. Although a fine pattern may give pure pattern EPs, a coarser version of the same pattern can give patterns responses mixed with EP components more or less similar to those elicited by flickering an unpatterned field^{29,37,38}. A curious consequence is that blurring a coarse pattern can increase the amplitude of pattern reversal though for fine patterns, as one would expect, blurring drastically attenuates the EP^{39,40}. More recently it has been suggested that the second types of EP component produced by pattern reversal reflect the activities of movement detectors rather than luminance responses^{26,41,42}.

The original evidence for pattern detectors tuned to narrow ranges of spatial frequency in the human visual system was psychophysical⁴³⁻⁴⁵, now supported by evidence from EPs to sinewave grating stimuli which are sensitive to grating orientation and spatial frequency¹². Suggestions,

based on psychophysical experiments that there exists a type of "feature filter", sensitive to an edge at some particular location in the visual field⁴⁶⁻⁴⁸ have been linked with the discovery of neurones in cat and monkey brains that are preferentially sensitive to light-dark boundaries in the visual field⁴⁹. Naturally, a sinewave grating covers a considerable area and also looks blurred at all times and would therefore not be expected to give EPs that emphasised the activities of local edge detectors. A pattern containing sharp edges, however, does elicit EPs that suggest some contribution from edge detectors. For example, EPs to a chequerboard pattern are almost abolished by occluding the edges of checks with very thin wires³⁷; and even very slight blurring markedly attenuates chequerboard EPs^{29,32,33,40}, a result that, because of EP saturation, would not be expected if the effect of blur were caused entirely by contrast reduction⁵⁰.

Colour coding of pattern vision

A pattern that is defined only in terms of colour with no luminance differences can give clear EPs. For example a fine pattern of alternate equiluminant red and green checks or stripes elicits EPs when the red and green elements are interchanged^{27,51} or when the pattern appears from a previously uniform yellow field⁵² (Fig. 3). Such EPs are markedly attenuated if the sharp edges of the bars or checks are even slightly blurred³³. These EPs are still large in the deliberately artificial situation when the eye's chromatic aberration is cancelled in such a way that both red and green images are sharply focused on to the retina⁵³. I have suggested that the existence of such EPs show that the physiological signals generated by red and green lights must still be segregated when they arrive at the most peripheral pattern-sensitive neurones in the eye (or brain). In other words, the most peripheral stage of pattern processing is

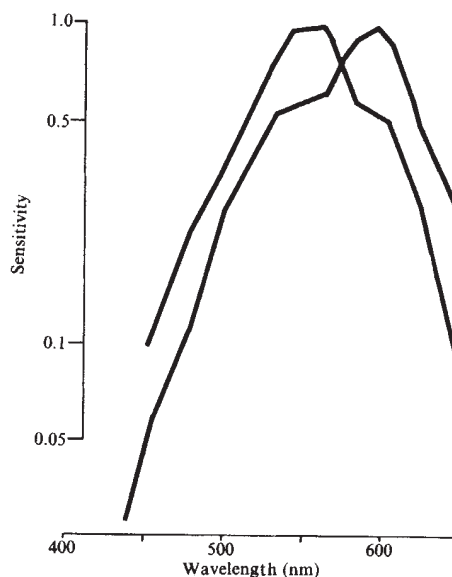


Fig. 5 Spectral sensitivities for the red and green channels of pattern vision. The red channel (predominantly) was stimulated by a foveal red (676 nm) pattern of fine checks as in Fig. 1, and a desensitising patch of light was superposed on the red pattern. The intensities of the desensitising patch needed for a given attenuation of the red pattern EP were plotted (reciprocally) as ordinates against desensitising wavelength, giving the right hand curve. The experiment was repeated with a green (544 nm) pattern to obtain an approximation to the spectral sensitivity of the green channel (left hand curve). These spectral sensitivities approximate those of Stiles π mechanisms. Note: because of the overlap of red and green spectral sensitivities, the colour channels are not completely separated by this technique. (Modified from Regan⁸.)

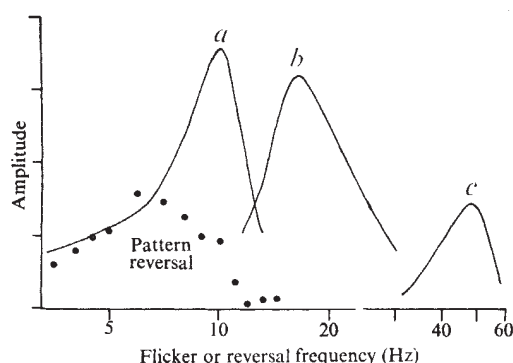


Fig. 6 *a*, Low; *b*, medium; and *c*, high frequency responses to flicker. The amplitudes of EPs produced by flickering an unpatterned patch of light show resonant-like peaks in three frequency regions. The properties of EPs are fairly uniform within a single frequency region, but are quite different in different frequency regions. EPs in the different frequency regions are generated in different areas of the brain, have different latencies, different colour properties and, furthermore, demyelinating disease slows medium frequency but not high frequency responses. The three types of signal may be separate from an early stage in the visual system. Note that these EPs to unpatterned flicker have quite different properties from EPs to fine patterns (whose frequency dependence is shown by dots). (Modified from Regan⁸⁶.)

duplicated in parallel red and green-sensitive channels (Fig. 4). There is evidence that segregation is total⁵². In effect these channels treat a fine pattern defined in terms of different colours as though it were defined by differences in light intensity across borders. Thus, neural responses to fine patterns (for example checks smaller than about 20 arc min) can be explained without invoking opponent colour mechanisms.

In the case of fine patterns, an interesting effect occurs when the relative intensities of red and green checks are critically adjusted. The sharp borders between the checks become indistinct^{54,56}, particularly when the eye's chromatic aberrations are cancelled⁵³. Significantly, red-green edges are minimally distinct when red and green luminances are equal⁵⁴⁻⁵⁶. Nevertheless, EPs to fine patterns are large at the balance point and show no sign of a minimum^{26,27,53}. This suggests that the red and green pattern channels, although separate at the most peripheral stage, fuse before the stage of perception in such a way that the resulting single pattern channel has the eye's photopic spectral sensitivity (Fig. 4).

Here the charm of EP recording is that a neural organisation is clearly revealed even though its operation is not subjectively evident and does not intrude into perception.

One function of this peripheral colour segregation might be to minimise the degradation of visual acuity caused by the eye's chromatic aberration. Red and green lights from a pattern are never simultaneously in sharp focus on the retina, so that a pattern detector sensitive to a wide range of colours would always receive a blurred retinal image. If, however, the most peripheral stage were segregated into two colour channels, then at any moment either the red or the green channel would receive a more sharply-focused image and preferentially feed into the next stage (Fig. 4). This would explain why visual acuity has been reported to be little worse in white than in monochromatic light^{57,60}, why acuity for one colour can be unaffected by superposing light of a different colour^{61-64,66}, why the eye's depth of focus is greater in white than in monochromatic light⁶⁸, and would also be in accord with the McCollough effect⁶⁵.

This is not the whole story, however. There seem to be additional detectors in the human brain that take advantage of colour differences in such a way that we see spatial patterns more clearly⁵³. These detectors respond preferentially to borders between differently coloured areas. Unlike

the intensity-pattern detectors described above, they are sensitive to chromatic contrast *per se* and may involve opponent colour mechanisms. Their activity is more evident for coarse than fine patterns¹²⁵.

Evoked potentials to a pattern of equiluminant red and green checks are a sensitive test for colour blindness⁵², and can give some insight into the condition. For example in one colour blind (deuteranopic) subject, EP amplitude could be completely abolished by carefully balancing the intensities of red and green checks⁵² (Fig. 3), which suggests that ocular chromatic aberration was almost ineffective⁵³ and therefore only one colour channel is present. Indeed in a later study¹¹, measurements of the pattern channel's action spectrum showed that the deuteranope investigated had a red mechanism only.

Approximations to the action spectra for the red and green channels of human pattern vision, measured objectively by recording EPs elicited by a pattern of fine checks directed on to the fovea⁸⁻¹¹, are similar to the action spectra of red and green retinal photopigments or the red and green colour mechanisms of Stiles (Fig. 5). In these experiments EP recording has the advantage over psychophysical methods that approximations to action spectra can be straightforwardly measured, not only near the threshold of pattern perception, but also at high, everyday levels of pattern sensation and with very little added adapting light. The point here is that if intense adapting lights are used, the effect of either red or green cones will be selectively reduced by chromatic adaptation so that separate colour mechanisms will be observed even if red and green signals are not segregated at the contrast stage. Only if very little adapting light is used can it be confirmed that red and green signals really are segregated at all times.

The blue channel of pattern vision is not evident when the eye is stimulated with a blue pattern, even when the

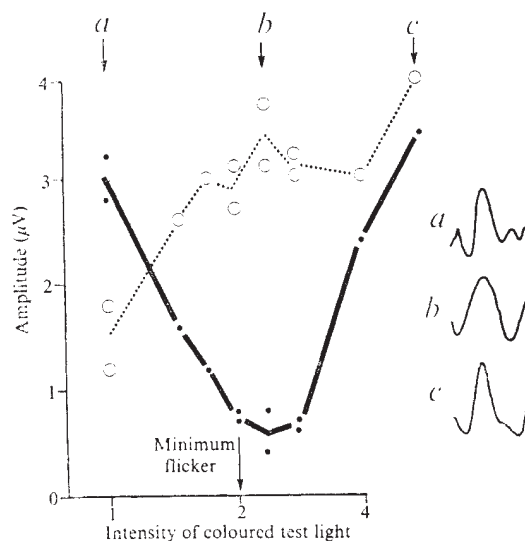


Fig. 7 A patch of standard white light was alternated with a patch of blue light 24 times/s. The blue light's intensity was adjusted by the subject until he saw minimum flicker (arrowed, 2 on abscissa). At this point, therefore, the blue test light and standard white light had the same photometric luminance. The steady-state EP was composed of two frequency components: —, a medium-frequency component of 24 Hz and ·····, a high-frequency component of 48 Hz. The high-frequency EP component fell to a minimum close to the point of minimum subjective flicker (continuous line), so that its amplitude correlated with the photometric luminance of the blue light. In contrast, the medium-frequency EP component (broken line) showed no minimum and was large even when the light did not appear to flicker at all (at arrow). This component therefore did not correlate with photometric luminance. Other evidence shows that it correlates with stimulus colour. Note that the averaged waveform (*a*, *b* and *c*) arbitrarily confounded the two components and was virtually uninterpretable. (Modified from Regan⁸⁴.)

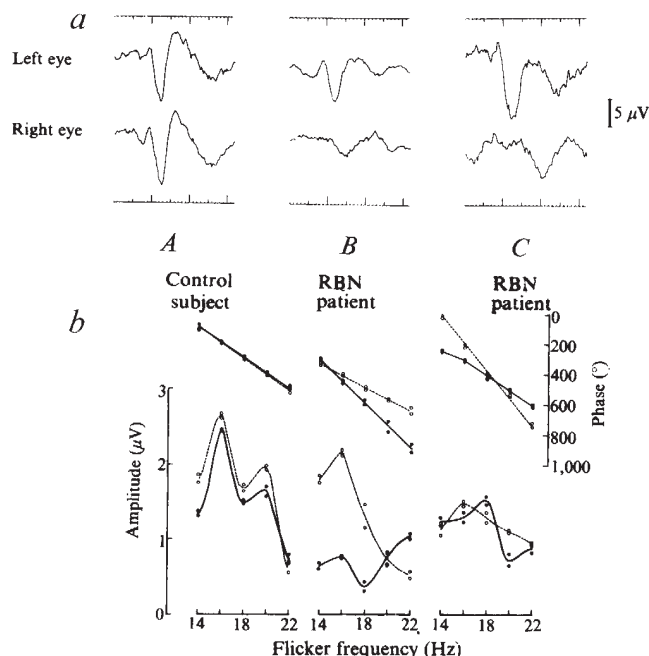


Fig. 8 Delayed visual evoked potential in patient with multiple sclerosis. *a*, Transient evoked potentials to pattern for a healthy subject (*A*) and two patients (*B*, *C*) who were recovering from acute attacks of retrobulbar neuritis in the right eye. Note the delayed and attenuated responses from the affected eyes. Time (abscissae) divided into 100, 50 and 10 ms. (From Halliday *et al.*⁸⁹) *b*, Steady-state evoked potentials elicited by flickering an unpatterned patch of light for a healthy control subject and for two retrobulbar neuritis (RBN) patients. —, The right eye; ---, the left eye. Note that for the affected eyes the plots of evoked potential phase against flicker frequency are steeper than for the good eyes (that is, the evoked potential is delayed for the affected eyes). This can be seen to hold independently of whether amplitude is attenuated. (From Milner *et al.*⁸⁷)

checks are as coarse as 40', presumably because the blue mechanism is insensitive. It can be revealed by using intense yellow chromatic adaptation in the same way that Stiles isolated his blue π mechanisms (ref. 67 and D.R., unpublished observations). More precise measurements on colour blind subjects have recently shown, that the blue pattern channel's action spectrum approximates that of Stile's blue π mechanism, even in the absence of chromatic adaptation.¹¹

The colour properties of pattern EPs described above are quite different from the colour properties of EPs elicited by unpatterned stimuli as described in the next section.

Unpatterned coloured stimuli

The aim of revealing differences between the ways in which the eye handles light of different colours has led several experimenters to compare EPs elicited by red, green, blue and yellow flashes. In experiments where different colours were reported to give different EP waveforms the stimulus fields seem to have been so large that they illuminated an inhomogeneous retinal region including rods as well as cones⁶⁸⁻⁷¹. In one crucial experiment stimulation was restricted to the fovea, a 2° central retinal area that is relatively homogeneous and mediates good colour vision⁷². This experiment did not support the notion that different colour sensations are associated with different EP waveforms, since red, yellow, green and blue flashes now gave transient EPs the waveforms of which were similar.

On the basis of EP latency rather than waveform Krauskopf was able to distinguish the separate contributions of red, green and (the slowest) blue chromatic mechanisms even when he used small (1°) foveal stimuli⁷³. Adapting Stiles' increment technique to EP work, Krauskopf used chromatic adaptation to select one or other chromatic

mechanism and obtained EP evidence that red, green and blue activities, separate at the most peripheral level, do not converge until at least 50 ms after stimulation. It has often been suggested that differences between the time courses of neural signals elicited by red, green and blue light may be the brain's physical representation of hue⁷⁴⁻⁷⁷. This notion might, perhaps, be open to test in man by further EP applications of Stiles' techniques for isolating colour mechanisms.

Although there seems to have been surprisingly little success in identifying EP correlates of the colour sensations aroused by monochromatic lights, the accompanying luminosity sensations seem to have clear EP correlates. Armington found that the amplitudes of some components of transient EPs to flash correlate with photometric luminance*, in other words they have the same spectral sensitivity as the light-adapted eye^{78,79}. Flickering an unpatterned patch of light gives steady-state EPs that are often composed of two frequency components, one at the flicker frequency and one at twice the flicker frequency. The two frequency components have quite different colour properties, provided that the frequency of one ("medium frequency") component falls in the range 13-25 Hz while that of the other ("high frequency") component fall in the range 35-60 Hz (Fig. 6). The amplitude of the high frequency component can be used to measure the spectral sensitivity curve of the eye with rapidity and a precision quite equal to that of the conventional psychophysical method (better than 0.05 log unit)^{34,81}. This method has already been used in goldfish⁸² and promises a simple alternative to behavioural methods in the monkey.

Although this high frequency component of the EP therefore correlates with photometric luminance, the medium frequency component does not, even though it is part of the same averaged waveform (Fig. 7). Medium frequency components do not even have a unique spectral efficiency curve⁸³, are very sensitive to chromatic adaptation^{81,84,85} and may reflect the activities of opponent colour mechanisms⁸⁶. The latencies of medium and high frequency EPs differ by about 2:1 (roughly 120 and 60 ms respectively)^{5,34,81,84,88}. High frequency and medium frequency EP components, carrying different visual information might well be anatomically separate, travelling along parallel channels, very early in the visual system (see below).

Clinical applications of evoked potentials

Two factors that initially prompted clinical application of EP recording were (1) the need for objective tests of sensory function in young children and babies where conventional ways of testing vision and hearing are uncertain, and (2) the failure of some EPs to correlate with sensation, which means that they offer access to some activities of sensory pathways not accessible to subjective tests. Both these possibilities have recently become practicalities, and it seems that indeed there are certain specific clinical diagnostic problems where custom-designed EP methods can usefully supplement conventional sensory tests.

One example is that of multiple sclerosis, in which breakdown of the insulating myelin sheath surrounding the axons (conducting fibres) of nerves eventually leads to impaired or blocked nervous conduction. This demyelination characteristically occurs at several sites in the central nervous system and multiple sclerosis can be difficult to diagnose when there is evidence of demyelination at only one site. This problem can be eased by using recently developed EP tests that detect damage to the visual pathway caused by retrobulbar neuritis, a common precursor of multiple sclerosis.

* "Photometric luminance" is not quite the same as "brightness". Even when intensity (and therefore photometric luminance) is held constant, the brightness of patterned stimulus can be altered by manipulating the patterning⁸⁰. In this manoeuvre, EP amplitude correlates with photometric luminance rather than brightness⁸⁰.

Transient EPs to pattern stimulation are significantly delayed in almost all patients after such an attack⁸⁹⁻⁹¹; steady-state EPs to pattern are also delayed^{87,88}. Flashing an unpatterned field, however, gives transient EPs that have been reported to provide no reliable indication of retrobulbar neuritis, though there is some disagreement on this point⁸⁹⁻⁹⁴. On the other hand flickering an unpatterned field elicits steady-state EPs that are reliably delayed in all patients for flicker frequencies in the medium-frequency region between 13 and 25 cs^{-1} (Figs 6 and 8)^{87,88}. The flicker test for retrobulbar neuritis has the advantage over the pattern test that it can be used even when the patient's visual acuity is low. Curiously, flicker EPs were delayed for no patients when flicker frequencies lay in the high-frequency region between 35 and 60 cs^{-1} ^{87,88}. This difference is an example of the ability of EPs to provide information not accessible to sensory tests, since neither medium-frequency nor high frequency EP amplitude correlates with flicker perception^{5,6,26,35}. The difference might be explained if the two types of flicker signal travel along different classes of axons in the optic nerve, one of which is preferentially demyelinated.

In a few patients delays may reach 100–180 ms⁸⁷⁻⁹¹. Such large delays are difficult to explain entirely in terms of slowed conduction along myelinated nerve fibres^{87,88}.

In patients in whom, on clinical evidence, demyelination seems restricted to the spinal cord, a high percentage have delayed transient EPs to pattern, and this evidence of visual pathology has proved useful in diagnosing multiple sclerosis^{89-91,95}. Interestingly, when steady-state EPs are recorded the flicker and pattern tests may pick up different patients, suggesting that the two tests are sensitive to different aspects of visual damage caused by multiple sclerosis⁹⁵.

Refraction and amblyopia

Spectacles can be prescribed by recording evoked potentials to pattern, since the largest responses are produced when the pattern looks sharpest^{7,96-101}. As little as one minute's recording is required when the power of the test lens is varied continuously during the examination⁷.

The clinician faces a more difficult problem when his patient cannot see fine detail, but spectacle lenses fail to improve vision. In this condition (amblyopia) the visual defect seems to lie in the networks of nerve cells in the retina and brain that handle pattern information. It seems likely that a period in early life during which a distorted image of the world is formed on the retina can be a major cause of this abnormal neural development. Childhood squint, and uncorrected long sightedness in one eye only are common causes of amblyopia¹⁰²⁻¹⁰⁴; uncorrected astigmatism can lastingly impair the ability to see straight lines of one particular orientation ("meridional amblyopia")^{108,107}. If allowed to persist until the child is too old, amblyopia becomes irreversible and the child may be left with only one good eye. During early childhood amblyopia can be cured by simple treatments such as by occluding the good eye for a period; normal vision then gradually returns to the amblyopic eye¹⁰²⁻¹⁰⁴. Accurate estimates of the patient's visual acuity are clearly important for effective treatment. Unfortunately it is during early childhood, the time when treatment is most effective, that reliable estimates of visual acuity are most difficult to obtain, especially in realistic conditions where several different retinal regions are simultaneously stimulated^{102,104}. Recording EPs to patterned stimuli offers a means not only of studying the mechanism of amblyopia but also of estimating the visual acuity of young children, and thus of improving treatment of amblyopia^{108,113} including meridional amblyopia^{7,114,115}.

There is disagreement whether stimulating the amblyopic eye's fovea gives normal EPs^{107,109-112}, but it does seem clear that the retinal area immediately surrounding the fovea (perifovea) give larger EPs for the amblyopic than

for the good eye¹¹⁰⁻¹¹². When fovea and perifovea are stimulated together (as in everyday life) foveal responses are relatively depressed¹¹⁰⁻¹¹² suggesting that different retinal regions interact abnormally. EPs from amblyopic and good eye are inverted in polarity, and this difference disappears after successful treatment¹¹².

If EP recording is to become a practical way of testing vision in young children it must be developed into a much briefer test. A speedy method developed with this in mind is to project on to a screen a checkerboard pattern whose dark and bright squares exchange places six times per second while slowly zooming up and down in size once every 30 s. The 6-Hz EP to the pattern is continuously plotted against check size, and several such graphs are electronically averaged. The child's attention is maintained during the 2–3 min required by projecting a cartoon film on to the checked pattern¹¹³.

Where binocular vision seems normal it is now possible to test stereoscopic depth vision objectively, since EPs can be recorded that are specific to changes in binocular disparity (that is, to movements in depth)^{116,117}.

Abnormal visual projections in albinos

In many animals including man the projection from each eye is divided so that signals from the right half of each retina go to the right hemisphere of the brain, and from the left hemiretina to the left hemisphere. Guillery and others have found that, in the albinos of several species, signals from a patch of retina near the midline are erroneously routed to the opposite hemisphere, so breaking up the orderly left-to-right projection of the retinal image on to the cortex.¹¹⁸⁻¹²² Guillery has suggested that the squint commonly found in these albinos may be a functionally useful adaptation to this misrouting.

Evoked potential recording offers one means of testing whether visual signals from part of the retina are routed to the wrong side of the brain in human albinos. When an albino subject viewed a visual stimulus with one eye only, the resulting EP seemed to be generated in one rather than both hemispheres^{123,124}. Although it is difficult to carry out visual EP tests on albinos with nystagmus, these results do indicate that EP recording offers a means of finding whether any patient with defective vision might have an underlying abnormality in the routing of his retino-cortical connections.

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- Dawson, G. D., *J. Physiol., Lond.*, **115**, 2–3 (1951).
- Dawson, G. D., *Electroenceph. clin. Neurophysiol.*, **6**, 65–84 (1954).
- Regan, D., thesis, Univ. London (1964).
- Regan, D., *Electroenceph. clin. Neurophysiol.*, **20**, 238–248 (1966).
- van der Tweel, L. H., and Verduyn Lunel, H. F. E., *Electroenceph. clin. Neurophysiol.*, **18**, 587–598 (1965).
- Regan, D., *Percept. Psychophys.*, **4**, 347–350 (1968).
- Regan, D., *Invest. Ophthalmol.*, **12**, 669–679 (1973).
- Regan, D., *Vision Res.*, **15**, 175–183 (1974).
- Regan, D., *Nature*, **250**, 437–439 (1974).
- Regan, D., *Proc. int. Symp. on EPs, Brussels* (edit. by Desmedt, J. D.) (in the press).
- Estevez, O., Spekreijse, H., van der Berg, T. J. T. P., and Cavonius, D., *Vision Res.* (in the press).
- Campbell, F. W., and Maffei, L., *J. Physiol., Lond.*, **207**, 635–652 (1970).
- Campbell, F. W., and Kulikowski, J. J., *J. Physiol., Lond.*, **222**, 345–356 (1972).
- Bryndahl, O., *J. opt. Soc. Am.*, **56**, 811–821 (1966).
- Fiorentini, A., and Maffei, L., *J. Physiol., Lond.*, **231**, 61–69 (1972).
- Campbell, F. W., Maffei, L., and Piccolino, M., *J. Physiol., Lond.*, **229**, 719–731 (1973).
- Bisti, S., and Maffei, L., *J. Physiol., Lond.*, **241**, 201–210 (1974).
- Maffei, L., and Fiorentini, A., *Vision Res.*, **13**, 1255–1267 (1973).
- Keidel, W. D., and Spreng, M., *Acta Oto-Laryng.*, **59**, 201–208 (1965).
- Keidel, W. D., and Spreng, M., *Internat. Audiol.*, **4**, 56–60 (1965).
- Davis, H., *Internat. Audiol.*, **5**, 77–81 (1966).
- Davis, H., Hirsh, S. K., Shelnutt, J., and Bowers, C., *J. Speech Hearing Res.*, **10**, 717–732 (1967).
- Rapin, I., and Graziani, L. J., *Neurol. (Minn.)*, **17**, 881–894 (1967).
- Rapin, I., in *Proc. int. Symp. on EPs, Brussels* (edit. by Desmedt, J. D.) (in the press).
- Kulikowski, J. J., and Kozak, W., in *Proc. 6th ISCEG Symp.*, 187–192 (Thieme, Leipzig, 1968).
- Regan, D., *Evoked Potentials in Psychology, Sensory Physiology and Clinical Medicine* (Chapman and Hall, London, and Wiley, New York, 1972).
- Regan, D., *Vision Res.*, **11**, 1203 (1971).

- ²⁸ Regan, D., in *The Visual System* (edit. by Arden, G. B.), 171-187 (Plenum, New York, 1972).
- ²⁹ Spekreijse, H., van der Tweel, L. H., and Zuidma, Th., *Vision Res.*, **13**, 1577-1601 (1973).
- ³⁰ Cobb, W. A., Ettlinger, G., and Morton, H. B., *Nature*, **216**, 1123-1125 (1967).
- ³¹ MacKay, D. M., *Nature*, **217**, 81-83 (1968).
- ³² van der Tweel, L. H., Regan, D., and Spekreijse, H., *Proc. 7th Int. Symp. ISCERG*, Istanbul, 1-11 (1969).
- ³³ Spekreijse, H., van der Tweel, L. H., and Regan, D., *Vision Res.*, **12**, 521-526 (1972).
- ³⁴ Regan, D., *J. opt. Soc. Am.*, **60**, 856-859 (1970).
- ³⁵ Regan, D., *Electroenceph. clin. Neurophysiol.*, **25**, 231-237 (1968).
- ³⁶ Regan, D., and Heron, J. R., *J. Neurol. Neurosurg. Psychiat.*, **32**, 479-483 (1969).
- ³⁷ Spekreijse, H., thesis, Junk, The Hague (1966).
- ³⁸ van der Tweel, L. H., and Spekreijse, H., *Ann. N.Y. Acad. Sci.*, **156**, 678-695 (1969).
- ³⁹ Regan, D., and Richards, W., *Vision Res.*, **11**, 679-684 (1971).
- ⁴⁰ Regan, D., and Richards, W., *J. opt. Soc. Am.*, **63**, 606-611 (1973).
- ⁴¹ Kulikowski, J. J., *J. Physiol., Lond.*, **242**, 40P (1974).
- ⁴² Kulikowski, J. J., in *Evoked Potentials* (edit. by Desmedt, J. D.) (in the press).
- ⁴³ Campbell, F. W., and Robson, J. J., *J. Physiol., Lond.*, **197**, 551-566 (1968).
- ⁴⁴ Blakemore, C., and Campbell, F. W., *J. Physiol., Lond.*, **203**, 237-260 (1969).
- ⁴⁵ Maffei, L., and Fiorentini, A., *Nature*, **240**, 479-481 (1972).
- ⁴⁶ Tolhurst, D. J., *Vision Res.*, **12**, 797-804 (1972).
- ⁴⁷ Shapley, R. M., and Tolhurst, D. J., *J. Physiol., Lond.*, **229**, 165-183 (1973).
- ⁴⁸ Kulikowski, J. J., and King-Smith, P. E., *Vision Res.*, **13**, 1455-1478 (1973).
- ⁴⁹ Hubel, D. H., and Wiesel, T. N., *J. Physiol., Lond.*, **148**, 574-591 (1959).
- ⁵⁰ Jeffreys, D. A., in *Proc. int. Symp. on EPs, Brussels* (edit. by Desmedt, J. D.) (in the press).
- ⁵¹ Regan, D., and Sperling, H., *Vision Res.*, **11**, 173-176 (1971).
- ⁵² Regan, D., and Spekreijse, H., *Vision Res.*, **14**, 89-95 (1974).
- ⁵³ Regan, D., *Vision Res.*, **13**, 2381-2402 (1973).
- ⁵⁴ Liebmman, S., *Psychol. Forsch.*, **9**, 300-353 (1972).
- ⁵⁵ Boynton, R. M., and Kaiser, P. K., *Science*, **161**, 366-368 (1968).
- ⁵⁶ Boynton, R. M., and Greenspon, T. S., *Vision Res.*, **12**, 495-507 (1972).
- ⁵⁷ Luckiesh, M., and Moss, F. K., *J. Franklin Inst.*, **215**, 401-410 (1933).
- ⁵⁸ Hartridge, H., *Recent Advances in the Physiology of Vision*, 1st ed. (Churchill, London, 1950).
- ⁵⁹ Hartridge, H., *Phil. Trans. Roy. Soc.*, **B232**, 519-561 (1947).
- ⁶⁰ Von Bahr, G., *Acta ophthalm.*, **24**, 129-146 (1946).
- ⁶¹ Campbell, F. W., and Gubisch, R. W., *J. Physiol., Lond.*, **192**, 345-358 (1967).
- ⁶² Green, D., *J. Physiol., Lond.*, **196**, 415-429 (1968).
- ⁶³ Sharp, C. R., *Vision Res.*, **14**, 41-51 (1974).
- ⁶⁴ McCollough, C., *Science*, **149**, 1115-1116 (1965).
- ⁶⁵ Campbell, F. W., *Optica Acta*, **4**, 157-164 (1957).
- ⁶⁶ Campbell, F. W., *Optica Acta*, **4**, 157-164 (1957).
- ⁶⁷ Stiles, W. S., *Proc. Roy. Soc.*, **B133**, 418-434 (1946); *Docum Ophthalm.*, **3**, 138-163 (1949); *Proc. natn. Acad. Sci. U.S.A.*, **45**, 100-114 (1959).
- ⁶⁸ Clynes, M., and Kohn, M., *Electroenceph. clin. Neurophysiol., Suppl.*, **1**, 82-96 (1967).
- ⁶⁹ Shipley, T., Jones, R. W., and Fry, A., *Science*, **150**, 1162-1164 (1965).
- ⁷⁰ Shipley, T., Jones, R. W., and Fry, A., *Vision Res.*, **6**, 657-667 (1966).
- ⁷¹ Yamanaka, T., Sobagaki, H., and Nayatani, Y., *Vision Res.*, **13**, 1319-1333 (1973).
- ⁷² Siegfried, J. B., *Am. J. Optom.*, and *Archiv. Am. Acad. Optom.*, **47**, 282-287 (1970).
- ⁷³ Krauskopf, J., *Vision Res.*, **13**, 2289-2298 (1973).
- ⁷⁴ Troland, L. T., *Am. J. Physiol. Opt.*, **2**, 23-48 (1921).
- ⁷⁵ Pieron, H., *Ann. Psychol.*, **32**, 1-29 (1932).
- ⁷⁶ Fry, G. A., *Am. J. Optom.*, **42**, 271-287 (1965).
- ⁷⁷ Donner, K. O., *Acta physiol. scand.*, **21**, Suppl. 72 (1950).
- ⁷⁸ Armington, J. C., *Docum. Ophthalm.*, **18**, 194-206 (1964).
- ⁷⁹ Armington, J. C., *Vision Res.*, **Suppl. 1**, 225-233 (1966).
- ⁸⁰ Cornsweet, T. N., *Visual Perception* (Academic Press, London, 1970).
- ⁸¹ Regan, D., *Neurosciences Res. Bull.*, **7**, 3 (1969).
- ⁸² Regan, D., Schellart, N. A. M., Spekreijse, H., and van der Berg, T. J. T. P., *Vision Res.* (in the press).
- ⁸³ Regan, D., *Vision Res.*, **10**, 163-178 (1970).
- ⁸⁴ Regan, D., *Vision Res.*, **8**, 149-158 (1968).
- ⁸⁵ Burkhardt, D. A., and Riggs, L. A., *Vision Res.*, **7**, 453-459 (1967).
- ⁸⁶ Regan, D., *Vision Res.*, **13**, 1933-1941 (1973).
- ⁸⁷ Milner, B. A., Regan, D., and Heron, J. R., *Brain*, **97**, 755-772 (1974).
- ⁸⁸ Heron, J. R., Regan, D., and Milner, B. A., *Brain*, **97**, 83-92 (1974).
- ⁸⁹ Halliday, A. M., MacDonald, W. I., and Mushin, J., *Lancet*, **i**, 982-985 (1972).
- ⁹⁰ Halliday, A. M., MacDonald, W. I., and Mushin, J., *Trans. Ophthalm. Soc. U.K.*, **93**, 315-324 (1973).
- ⁹¹ Halliday, A. M., MacDonald, W. I., and Mushin, J., *Proc. int. Symp. on EPs, Brussels* (edit. by Desmedt, J. D.) (1974).
- ⁹² Richey, E. T., Kooi, K. A., and Tourtellotte, W. W., *J. Neurol. Neurosurg. Psychiat.*, **34**, 275-280 (1971).
- ⁹³ Namerow, N. S., and Enns, N., *J. Neurol. Neurosurg. Psychiat.*, **35**, 829-833 (1972).
- ⁹⁴ Feinsod, M., Abramsky, O., and Auerbach, E., *J. Neurol. Sci.*, **20**, 161-175 (1973).
- ⁹⁵ Regan, D., Milner, B. A., and Heron, J. R., in *Proc. int. Symp. on EPs, Brussels*, (edit. by Desmedt, J. D.) (1974).
- ⁹⁶ Harter, M. R., and White, C. T., *Vision Res.*, **8**, 701-711 (1968).
- ⁹⁷ Millodot, M., and Riggs, L. A., *Arch. Ophthalm.*, **84**, 272-278 (1970).
- ⁹⁸ Duffy, F. H., and Rengstorff, R. H., *Am. J. Optom.*, **48**, 717-728 (1971).
- ⁹⁹ Dawson, W. W., Perry, N. W., and Childers, D. G., *Invest. Ophthalm.*, **11**, 789-799 (1972).
- ¹⁰⁰ Ludlam, W., and Myers, R. R., *Trans. N.Y. Acad. Sci.*, **34**, 154-170 (1972).
- ¹⁰¹ McCormack, G., and Marg, E., *Am. J. Optom.*, **50**, 889-903 (1973).
- ¹⁰² Shapiro, M., *Amblyopia* (Chilton, New York, 1971).
- ¹⁰³ Lyle, T. K., and Wybar, K. C., *Practical Orthoptics in the Treatment of Squint* (Lewis, London, 1970).
- ¹⁰⁴ Reinecke, R. D., *Arch. Ophthalm.*, **91**, 501-514 (1974).
- ¹⁰⁵ Lombroso, C., Duffy, F., and Robb, R., *Electroenceph. clin. Neurophysiol.*, **27**, 238-247 (1969).
- ¹⁰⁶ Freeman, R. D., Mitchell, D. E., and Millodot, M., *Science*, **175**, 1384-1368 (1972).
- ¹⁰⁷ Mitchell, D. E., Freeman, R. D., Millodot, M., and Haegerstrom, G., *Vision Res.*, **13**, 535-588 (1973).
- ¹⁰⁸ Yinon, U., Jakobovitz, L., and Auerbach, E., *Invest. Ophthalm.*, **13**, 293-296 (1974).
- ¹⁰⁹ Spekreijse, H., Khoe, L. H., and van der Tweel, L. H., *Adv. Exp. Biol. Med.*, **24**, 141-156 (1972).
- ¹¹⁰ Sokol, S., and Bloom, B., *Invest. Ophthalm.*, **12**, 936-939 (1973).
- ¹¹¹ Sokol, S., in *Proc. int. Symp. on EPs, Brussels* (edit. by Desmedt, J. D.) (1974).
- ¹¹² Arden, G. B., *Proc. Roy. Soc. Med.*, **66**, 1037-1043 (1973).
- ¹¹³ Regan, D., in *Proc. int. Symp. on EPs, Brussels* (edit. by Desmedt, J. D.) (1974).
- ¹¹⁴ Fiorentini, A., and Maffei, L., *Vision Res.*, **13**, 1781-1783 (1973).
- ¹¹⁵ Freeman, R. D., and Thibos, L. N., *Science*, **180**, 876-878 (1973).
- ¹¹⁶ Regan, D., and Spekreijse, H., *Nature*, **255**, 92-94 (1970).
- ¹¹⁷ Regan, D., and Beverley, K. I., *Nature*, **246**, 504-506 (1973).
- ¹¹⁸ Guillery, R. W., *Brain Res.*, **14**, 739-741 (1969); **33**, 482-485 (1971).
- ¹¹⁹ Guillery, R. W., Amorn, C. S., and Eighmy, B. B., *Science*, **174**, 831-832 (1961).
- ¹²⁰ Guillery, R. W., Scott, G. L., Caltanach, B. M., and Dale, M. S., *Science*, **179**, 1014-1016 (1973).
- ¹²¹ Hubel, D. H., and Wiesel, T. N., *J. Physiol., Lond.*, **218**, 33-62 (1971).
- ¹²² Berman, N., and Cyander, M., *J. Physiol., Lond.*, **224**, 363-389 (1972).
- ¹²³ Regan, D., *Proc. 11th Int. ISCERG Symp.*, *Bad. Neuheim*, 1973 *Docum Ophthalm.*, **285-301** (1974).
- ¹²⁴ Creel, D., Witkop, C. J., and King, R. A., *Invest. Ophthalm.*, **13**, 430-440 (1974).
- ¹²⁵ DeValois, R. L., *Invest. Ophthalm.*, **11**, 417-427 (1972).

articles

Surface reactivity of tungsten

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Pre-adsorbed nitrogen or β -carbon monoxide inhibit the surface reactivity of tungsten towards hydrogen, both in catalysis and in adsorption. The effect of nitrogen can be satisfactorily accounted for by a simple reduction in the area of reactive filament, taking into account the selective adsorption of this gas by the low index planes of tungsten. With carbon monoxide the effect is considerably greater, perhaps because of lateral interactions between adsorbed hydrogen and carbon monoxide.

ISOTOPE equilibration reactions taking place on the surface of a metal are among the simplest examples of heterogeneous

catalysis and thus provide a suitable starting point for investigating in detail the catalytic process. One particularly attractive feature of the study of these reactions is the excellence of the agreement observed between experiments carried out on polycrystalline wires of quite different origin¹⁻⁶. In addition to the intrinsic interest of such studies, it is possible to use the equilibration reaction as a chemical probe to investigate the surface. Thus, when a tungsten wire was used as a catalyst for nitrogen, carbon monoxide or hydrogen isotope equilibration^{4,6,7} it was found that poisoning by pre-adsorbed oxygen was nearly linearly dependent on the fractional oxygen coverage. From this it was inferred that active patches⁸ did not form a significant part of the surface. Furthermore, combination of the results of these experiments with measurements of the adsorptive characteristics of the surface (the saturation uptake and the initial

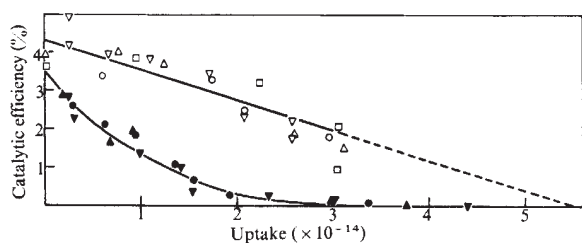


Fig. 1 Inhibition of hydrogen isotope equilibration at room temperature by adsorbed nitrogen ($\circ$, ∇ , Δ , $\square$) and by adsorbed carbon monoxide ($\blacktriangle$, $\bullet$, $\blacktriangledown$). Total pressure of hydrogen isotopes ($\times 10^8$): $\square$, 5.6; ∇ , 25; $\circ$, 28; Δ , 61; $\blacktriangle$, 5.1; $\bullet$, 24; $\blacktriangledown$, 26. Pumping constant for HD: $0.48 \pm 0.07 \text{ s}^{-1}$. Uptakes measured as atoms cm^{-2} for nitrogen and molecules cm^{-2} for carbon monoxide.

sticking probability) shed light on the close, yet elusive, relationship between the centres of catalysis and chemisorption. Some further evidence on this point has come from studies of the catalytic activity of the low index planes of tungsten [(100), (110), and (111)] in ammonia decomposition⁹, in which it was reported that the activities of the planes were different, as are their reactivities towards nitrogen¹⁰.

Because nitrogen and carbon monoxide are both more strongly bonded in their β states to polycrystalline tungsten than is hydrogen, these gases might be expected to inhibit the activity of tungsten as a hydrogen isotope equilibration catalyst. If this were to happen it would then be possible to compare the effects of a dissociated-molecule inhibitor¹⁰ (N_2) with the probably, though not certainly, undissociated carbon monoxide¹⁰⁻¹². It is this comparison we report here.

Measuring catalytic activity

The same apparatus was used as for the earlier experiments⁴. It consists of a Pyrex glass ultra-high vacuum (UHV) chamber ($p_{\text{min}} < 5 \times 10^{-10}$ torr) in which the experimental filament (length 20.7 cm and geometric area 0.83 cm^2) is suspended. The filament had been cleaned previously by extensive heating in oxygen and its reactivity was monitored regularly during the present series of experiments, by observing the uptake and initial sticking probability of nitrogen. The constancy of these quantities during the course of the work was taken as sufficient evidence that the filament did not become contaminated. Flashing to about 2,040 K *in vacuo* sufficed to remove adsorbed gases and was the normal method of cleaning.

The experimental gases were admitted through all-metal valves from either of two gas storage volumes. Adjustment of the apertures of these valves produced the required partial pressure of a gas. A similar valve connecting the UHV chamber

to the pump controlled the rate of flow of a gas over the filament. Partial pressures were measured with an omegatron radio-frequency mass spectrometer which formed part of the UHV section. A commercial Bayard-Alpert ionisation gauge (Edwards High Vacuum) with a low temperature, lanthanum hexaboride-coated rhenium filament was used to calibrate the mass spectrometer. The catalytic activity of the filament was measured by streaming a mixture of approximately equal partial pressures of hydrogen and deuterium over it and observing the formation of HD at the expense of H_2 and D_2 .

The efficiency E of the filament as an equilibration catalyst has been defined previously⁷ by the ratio

$$E = \frac{\text{Observed rate of production of HD}}{\text{Maximum possible rate of production of HD}}$$

The way in which the heights of the mass spectrometer peaks, recorded under steady state conditions, are used to calculate E

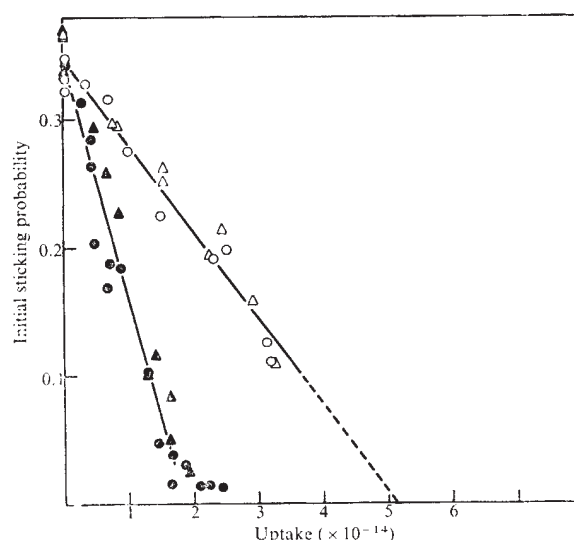
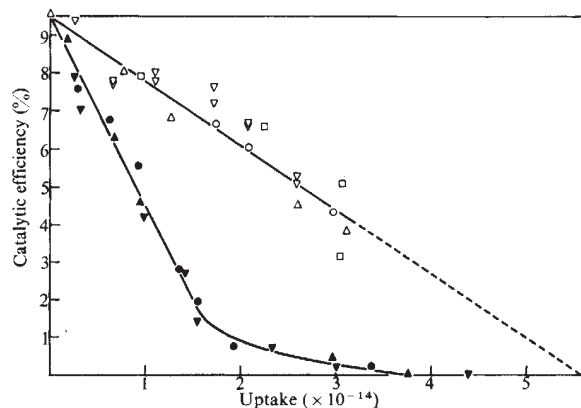


Fig. 3 Effect of pre-adsorbed nitrogen or carbon monoxide on s_0 of hydrogen ($\circ$ or $\bullet$) or deuterium (Δ or $\blacktriangle$). Uptakes of nitrogen or carbon monoxide as in Fig. 1.

has been fully described elsewhere⁷. Earlier experiments have shown that E is independent of pressure within the range 2×10^{-7} – 9×10^{-7} torr (ref. 7), and a total pressure between 5×10^{-8} and 6×10^{-7} torr was used in these experiments. The catalytic inhibiting effect of pre-adsorbed nitrogen or carbon monoxide was investigated as follows. The filament was cleaned and cooled to room temperature *in vacuo*. Nitrogen or carbon monoxide was then let in and the filament was allowed to adsorb for the appropriate time. After cutting off the gas supply the filament was heated briefly to 650 K to equilibrate the adsorbed layer and to remove any gas in the weaker α state¹¹. The hydrogen–deuterium mixture was then streamed over the filament at a chosen temperature and when the peak heights of masses 2, 3 and 4 were constant they were recorded. Contamination of the filament by the background gas (largely CO) presented more of a problem in this work than it had done previously, because it was not possible to flash off impurities accumulated from the background without disturbing the initial layer. This difficulty was particularly marked with CO which is reversibly adsorbed by the walls of the UHV chamber^{13,14}. The experimental time for measurements of catalytic activity at any particular coverage was thus limited to about 0.5 h. After the measurements had been made the uptake of carbon monoxide was determined by heating the filament in stages; to 650 K to remove the hydrogen and, when the gas had pumped away, to 1,610 K to desorb carbon

Fig. 2 Inhibition of hydrogen isotope equilibration at elevated temperatures by adsorbed nitrogen (800 K) and by adsorbed carbon monoxide (690 K). Symbols as in Fig. 1.



monoxide. A record of the pressure burst caused by this desorbed gas was made with a rapid-response amplifier and ultraviolet recorder, from which record the uptake was calculated. Nitrogen uptakes were calculated from the partial pressure record, made with the omegatron, during the initial adsorption¹⁵.

The effects of pre-adsorbed nitrogen or carbon monoxide on the adsorptive characteristics of the filament, as defined by the initial sticking probability s_0 and the maximum uptake θ_{H_2} at a pressure near 1×10^{-7} torr, were determined in a separate series of experiments. To do this the filament was partially covered as before and hydrogen or deuterium was admitted at a constant rate from a storage volume. The partial pressure during the adsorption period was recorded with the omegatron until the filament was saturated. s_0 and θ_{H_2} were both calculated from this record¹⁵.

The effects of the two gases on the efficiency at room temperature are shown in Fig. 1 and in the plateau region⁷ (690 K for CO, 800 K for N_2) in Fig. 2. Within a set of experiments carried out under fixed conditions the relative values of E are consistent, though the absolute accuracy is not better than $\pm 25\%$ (ref. 7). The ways in which s_0 and θ_{H_2} depend on the uptakes of CO or N_2 are shown in Figs 3 and 4.

Implications

The discussion of these results and their relationship to the earlier observations of the effect of pre-adsorbed oxygen atoms on the surface reactivity of this filament towards nitrogen, hydrogen and carbon monoxide gives an opportunity of reviewing the conclusions that can be drawn from experiments of this type about the chemical constitution of the surface. In so doing it is appropriate to relate the work, where possible, to the conclusions drawn from other techniques. But, in view of the proliferation of papers relating to the surface of tungsten the comparison must be selective.

We shall take as the starting point for discussion a consideration of the relevant geometric factors. Tungsten has the b.c.c. structure and it has been suggested that a well annealed polycrystalline filament will expose predominantly the (100) and (110) planes^{16,17}. As far as the influence of nitrogen on the catalytic efficiency is concerned, this suggestion provides an immediate interpretation of the considerable remaining catalytic activity of the nitrogen-saturated filament, roughly half that of the clean filament, because nitrogen adsorption on tungsten shows a strong surface-plane dependence. A careful review of the evidence reached the conclusion that dissociative chemisorption occurs on W(100) at room temperature, but that the W(110) plane is unreactive¹⁰. Subsequent experiments have either confirmed this conclusion^{18,19} or have demonstrated that, at most, adsorption takes place with a sticking probability which is initially very low (0.005) and which thereafter declines rapidly²⁰. On the other hand hydrogen shows no such geometric sensitivity and adsorbs readily and extensively on a polycrystalline tungsten filament^{7,16,21,22} and on both of these single crystal planes²³⁻²⁶. The catalytic activity towards hydrogen of the nitrogen-free surface can thus be attributed to both (100) and (110) planes. Saturation with nitrogen then removes the reactive sites on (100) planes but leaves the (110) planes clean and still reactive.

The near-linear decline in efficiency with increasing nitrogen uptake is closely paralleled by the fall in s_0 and θ_{H_2} . In this, the effect of nitrogen closely resembles the way in which the first stage of oxygen adsorption reduces the activity of this filament towards hydrogen (though oxygen will eventually eliminate all reactivity) and the results can be interpreted (as for O-atom adsorption) as the consequence of a reduction in the area of reactive surface directly proportional to the uptake of the pre-adsorbed gas. The similarity between oxygen and nitrogen extends further, in that for both gases the reactivity is halved by a coverage of about 2.7×10^{14} atoms cm^{-2} . But, whereas for nitrogen all the atoms are on the (100) plane, the non-selective adsorption of oxygen by tungsten²⁷⁻²⁹ presumably leads to a

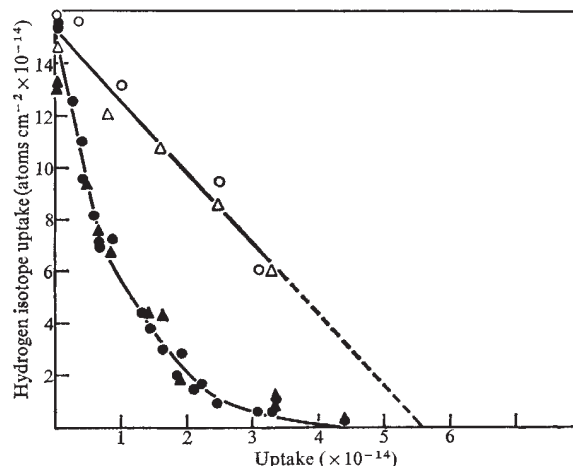


Fig. 4 Effect of pre-adsorbed nitrogen or carbon monoxide on maximum room-temperature uptake of hydrogen ($\circ$ or $\bullet$) or deuterium ($\triangle$ or $\blacktriangle$). Uptakes of nitrogen or carbon monoxide as in Fig. 1.

distribution of oxygen atoms over both the (100) and (110) planes. It is interesting to note that this chemical similarity between adsorbed oxygen and nitrogen is reflected in the formation of ordered geometric arrangements, as detected by LEED (low energy electron diffraction), of the atoms of both gases on the (100) plane^{30,31}.

There are two previous experiments concerned with the co-adsorption of nitrogen and hydrogen on tungsten which are of particular relevance to this work^{21,32}. These experiments were principally concerned with the uptakes resulting from sequential adsorption of nitrogen and hydrogen and with the ability of nitrogen to replace previously adsorbed hydrogen on either a polycrystalline wire²¹ or a W(100) single crystal plane³². The major conclusion drawn from both of the earlier experiments, and one with which the results presented here are in complete accord, was that there is very little interaction between adsorbed hydrogen and adsorbed nitrogen.

We now turn to a consideration of the effect of pre-adsorbed carbon monoxide on the reactivity of tungsten. The method used for preparing a surface partially covered with CO ensured that the gas was held in the more strongly bonded of the surface states of CO on tungsten, the β states, the α -CO having been desorbed by the prior heating to 650 K (ref. 33). The β state is itself composite and consists of three substates, normally called β_1 , β_2 and β_3 in ascending order of binding strength. As expected these were poorly resolved under the conditions of our experiments¹⁴ though with special care separation is possible³³. In adsorption, the β_2 and β_3 states fill up first so that the early part of the catalytic inhibition curve is associated with the poisoning effect of carbon monoxide in these states. As Figs 1 and 2 show $(\beta_2 + \beta_3)$ -CO has a profound effect on the reactivity of tungsten; an uptake which is only about 40% of β -CO saturation reduces E by about 90%. This uptake has a comparable effect on s_0 and θ_{max} as Figs 3 and 4 show. It is thus clear that a CO molecule is much more effective at deactivating the surface than either an oxygen atom or a nitrogen atom. This observation again agrees very satisfactorily with the earlier measurements of the co-adsorption of carbon monoxide and hydrogen^{21,34}. The more recent of these studies showed that adsorption of CO weakened the bonding of hydrogen to the surface (in this case the (100) plane) thereby generating a new state which was desorbed at 273 K but which was retained on the surface at lower temperatures. A fractional coverage of CO equal to 0.4 was sufficient to exclude all β -hydrogen from the surface at 273 K. Bearing in mind that this observation relates to a monocrystalline surface and the results in Figs 1-4 are for a polycrystalline surface, the concordance between the two sets of experiments is striking. It has been pointed out, however,

that the characteristic desorption spectrum of β -CO from tungsten can be accounted for satisfactorily starting from the postulate that carbon monoxide adsorption takes place dissociatively and that lateral, repulsive interactions are present between the adsorbed atoms³⁵.

The initial purpose of this series of experiments was an attempt to locate the sites of catalytic activity on a tungsten surface. There seems to be little doubt that for the reactions chosen to probe the surface, which probably come into the category of 'facile'³⁶, much of the surface which is capable of adsorbing the gases is also catalytically active. It will be interesting to discover whether this is also true of more complex reactions.

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¹ Madey, T. E., and Yates, J. T., Jr, *J. chem. Phys.*, **51**, 345 (1969).

² Boon, C., Gasser, R. P. H., and Tovey, H., *Trans. Faraday Soc.*, **65**, 3111 (1969).

³ Madey, T. E., and Yates, J. T., Jr, *J. chem. Phys.*, **44**, 1675 (1966).

⁴ Gasser, R. P. H., and Vaught, P. R., *Nature*, **221**, 166 (1969).

⁵ Madey, T. E., and Yates, J. T., Jr, *Proc. sixth Rarefied Gas Dynamics Symp.*, 1968.

⁶ Gasser, R. P. H., and Vaught, P. R., *Nature*, **225**, 933 (1970).

⁷ Gasser, R. P. H., Morton, T. N., Overton, J. M., and Ward, M. J., *Nature*, **239**, 29 (1972).

⁸ Rideal, Eric K., *Concepts in Catalysis* (Adademic Press, London and New York, 1968).

⁹ McAllister, J., and Hansen, R. S., *J. chem. Phys.*, **59**, 414 (1973).

¹⁰ Ehrlich, G., *A. Rev. phys. Chem.*, **17**, 295 (1966).

¹¹ Ford, R. R., *Adv. Catalysis*, **21**, 51 (1970).

¹² Goymour, C. G., and King, D. A., *J. chem. Soc. Faraday Trans. I*, **69**, 736 (1973).

¹³ Hobson, J. P., and Earnshaw, J. W., *J. Vac. Sci. Technol.*, **4**, 257 (1967).

¹⁴ Ehrlich, G., *J. chem. Phys.*, **34**, 39 (1961).

¹⁵ Eisinger, J., *J. chem. Phys.*, **29**, 1154 (1958).

¹⁶ Eley, D. D., and Norton, P. R., *Proc. R. Soc.*, **A314**, 301 (1970).

¹⁷ Cartier, P. G., and Rye, R. R., *J. chem. Phys.*, **56**, 5316 (1972).

¹⁸ van Oostrom, A., *J. chem. Phys.*, **47**, 761 (1967).

¹⁹ King, D. A., and Wells, M. G., *Surf. Sci.*, **29**, 454 (1972).

²⁰ Tamm, P. W., and Schmidt, L. D., *Surf. Sci.*, **26**, 286 (1971).

²¹ Rigby, L. J., *Can. J. Phys.*, **43**, 1020 (1965).

²² Ricca, F., Medana, R., and Saini, G., *Trans. Faraday Soc.*, **61**, 1492 (1965).

²³ Madey, T. E., *Surf. Sci.*, **36**, 281 (1973).

²⁴ Tamm, P. W., and Schmidt, L. D., *J. chem. Phys.*, **54**, 4775 (1971).

²⁵ King, D. A., and Menzel, D., *Surf. Sci.*, **40**, 399 (1973).

²⁶ Tamm, P. W., and Schmidt, L. D., *J. chem. Phys.*, **55**, 4253 (1971).

²⁷ Hopkins, B. J., and Pender, K. R., *Surf. Sci.*, **5**, 155 (1966).

²⁸ Bell, A. E., Swanson, L. W., and Crouser, L. C., *Surf. Sci.*, **10**, 254 (1965).

²⁹ Vas'ko, N. P., Ptushinskii, Y. G., and Chuikov, B. A., *Surf. Sci.*, **14**, 448 (1969).

³⁰ Adams, D. L., and Germer, L. H., *Surf. Sci.*, **26**, 109 (1971).

³¹ Anderson, J., and Danforth, W. F., *J. Franklin Inst.*, **279**, 160 (1965).

³² Yates, J. T., Jr, and Madey, T. E., *J. Vacuum Sci. Technol.*, **8**, 63 (1971).

³³ Redhead, P. A., *Trans. Faraday Soc.*, **57**, 641 (1961).

³⁴ Yates, J. T., Jr, and Madey, T. E., *J. chem. Phys.*, **54**, 4969 (1971).

³⁵ Goymour, C. G., and King, D. A., *J. chem. Soc. Faraday Trans. I*, **69**, 749 (1973).

³⁶ Boudart, M., *Adv. Catalysis*, **20**, 153 (1969).

The nucleotide sequence of an RNA polymerase binding site on bacteriophage fd DNA

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The nucleotide sequence of the RNA polymerase binding site at a promoter on fd RF DNA has been determined in comparison with the starting sequence of RNA initiated at this promoter. The RNA polymerase binding site contained the startpoint of transcription in the centre and a region with twofold symmetry in the non-transcribed part.

SEVERAL species of RNA with unique starting sequences and sizes are transcribed on the doubly closed replicative form (RF-I) DNA of fd bacteriophage¹. This suggests that fd RF-I DNA provides sets of specific sites for initiation and termination of transcription. To analyse the structure at such an RNA initiation site (promoter), we first constructed the cleavage map of RF-I DNA using *Haemophilus* restriction endonucleases, and then localised the promoters on the map. We found that a short DNA segment, Hap-Hga V, contained one of the promoters for G-start RNA (Fig. 1), the fragment representing about 3.8% of the length of fd DNA and corresponding to about 230 base pairs. A uniform RNA of about 120 bases long was efficiently synthesised on this fragment. The DNA fragment was specifically bound to RNA polymerase with GTP present. As the binding of the polymerase to DNA renders the covered portion of the DNA resistant to DNase^{2,3}, the region protected by the polymerase was isolated from Hap-Hga V. We compared the nucleotide sequence of the polymerase binding site isolated in this way with the sequence of RNA initiated on Hap-Hga V, based on the transcription of the DNA sequence into RNA in the oligonucleotide-primed reaction⁴.

Preparation of fd RF-I DNA, *E. coli* RNA polymerase, and restriction endonucleases (Hap and Hga) from *H. aphrophilus* and *H. gallinarum* was as previously described^{5,6}. For preparation of Hap-Hga V, a complete digest of RF-I DNA with Hap and Hga was prepared⁵, mixed with RNA polymerase and GTP; DNA fragments which formed stable complexes with the

polymerase were isolated as in the legend to Fig. 2b. The two fragments (Hap C and Hap-Hga V in Fig. 2b) isolated by this

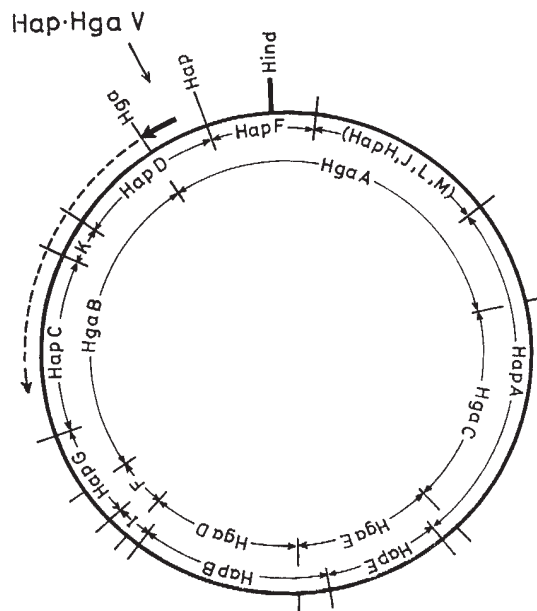


Fig. 1 The location of Hap-Hga V on the cleavage map of fd DNA. The map has been constructed using the cleavages with Hap, Hga and Hind (a restriction endonuclease from *H. influenzae* Rd). Hap A,B — — — and Hga A,B — — — label of fragments created by Hap and Hga (our unpublished observations). Hap-Hga V is produced either by digestion of Hap D with Hga or by digestion of Hga A with Hap. The G-start RNA initiated on Hap-Hga V proceeds in the counter-clockwise direction and terminates at a site on Hap C.

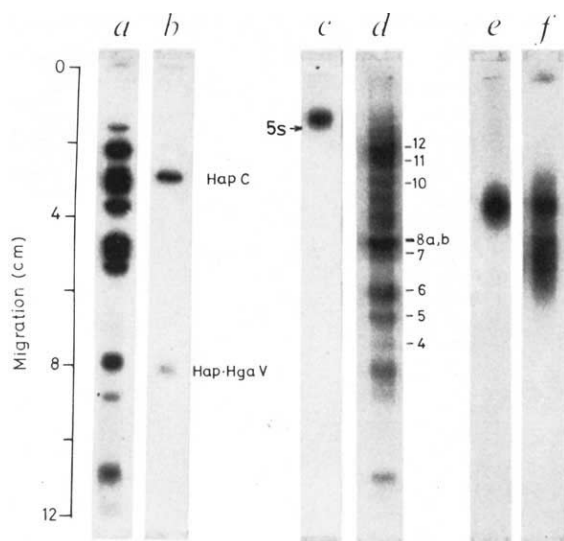


Fig. 2 Gel electrophoretic patterns of (a) a digest of fd RF-I DNA with *Hap* and *Hga*, (b) DNA fragments which bind to RNA polymerase with GTP present, (c) RNA synthesised on Hap-Hga V, (d) a partial T₁ RNase digest of RNA made on Hap-Hga V, (e) the RNA polymerase binding site isolated from Hap-Hga V, and (f) (A)₃ U-primed RNA formed on the polymerase binding site. (a) A complete digest of ³²P-RF-I DNA with *Hap* and *Hga* was layered on a 5% polyacrylamide gel column (0.6 cm × 12 cm), electrophoresed for 12 h at 2 mA per tube, and the autoradiograph was taken as in ref. 5. (b) The Hap-Hga digest of ³²P-RF-I DNA (0.1 mg) was added to a binding mixture (3 ml) containing 8 mM MgCl₂, 50 mM KCl, 40 mM Tris (pH 7.9), 0.1 mM GTP, and about 100 pmol RNA polymerase. After 20 min at 30° C, the mixture was passed through a Millipore filter (HA 0.45 μm). The filter was washed with 1 M KCl, 8 mM MgCl₂, 20 mM Tris (pH 7.9) and then with 8 mM MgCl₂, 20 mM Tris (pH 7.9). DNA fragments retained on the filter were eluted with 0.2% SDS-20 mM Tris (pH 7.9). The eluate was deproteinised, concentrated and electrophoresed as in (a). (c) Hap-Hga V (non-labelled, about 2 pmol) was added to a reaction mixture (1 ml) containing 8 mM MgCl₂, 50 mM KCl, 40 mM Tris (pH 7.9), 0.1 mM dithiothreitol, 0.1 mM α³²P-GTP (10⁷ c.p.m. nmol⁻¹), 0.2 mM each of three other nucleotide triphosphates, and about 10 pmol RNA polymerase. Incubation for 30 min at 37° C was terminated by shaking with 80% phenol. The aqueous layer was passed through a Sephadex G50 column (1 cm × 30 cm). The RNA fraction was collected, and electrophoresed on a 15% gel column for 3 h at 150 V as in ref. 7. Under these conditions, about 7 pmol of RNA were synthesised. Arrow shows position of 5S ribosomal RNA as marker. (d) ³²P-RNA synthesised on Hap-Hga V was partially digested with T₁ RNase as in ref. 6, and the digest was electrophoresed as in (c). The numbered bands were extracted, rerun on 15% gels containing 7 M urea, and submitted to sequence analysis. (e) Hap-Hga V (labelled with ³²P, about 20 pmol) was added to the binding mixture given in (b). After 10 min at 30° C, 50 μg of DNase I were added and incubation was continued for an additional 30 min. Digestion was terminated by shaking with 80% phenol. The aqueous layer was treated with ethylether, dialysed against 0.1 mM EDTA-20 mM Tris (pH 7.9), and electrophoresed on a 15% gel as in (c). (f) The portion of Hap-Hga V protected from DNase I digestion (about 4 pmol) was heated for 5 min at 100° C, rapidly cooled, and added to a reaction mixture (1 ml) containing 8 mM MgCl₂, 50 mM KCl, 40 mM Tris (pH 7.9), 0.1 mM dithiothreitol, 10 μM α³²P-GTP (5 × 10⁷ c.p.m. nmol⁻¹) and 20 μM each of three other nucleotide triphosphates, 10 μmol AAAU, and about 10 pmol of RNA polymerase. Incubation was carried out for 3 h at 37° C, and terminated by shaking with 80% phenol. After removal of phenol with ethylether, the template was digested with DNase I. The solution was deproteinised, and passed through a Sephadex G-50 column. The RNA fraction collected was mixed with 50 μg of fd SS DNA. NaCl was added to 0.9 M and sodium citrate to 0.09 M. The mixture was heated for 5 min at 100° C, held for 3 h at 51° C, slowly cooled, and passed through a Millipore filter. The filter was washed with 0.3 M NaCl, 0.03 M sodium citrate. RNA retained on the filter was dissociated by heating for 5 min at 100° C in 20 mM Tris (pH 7.9), and electrophoresed on a 15% gel column containing 7 M urea, as in (c).

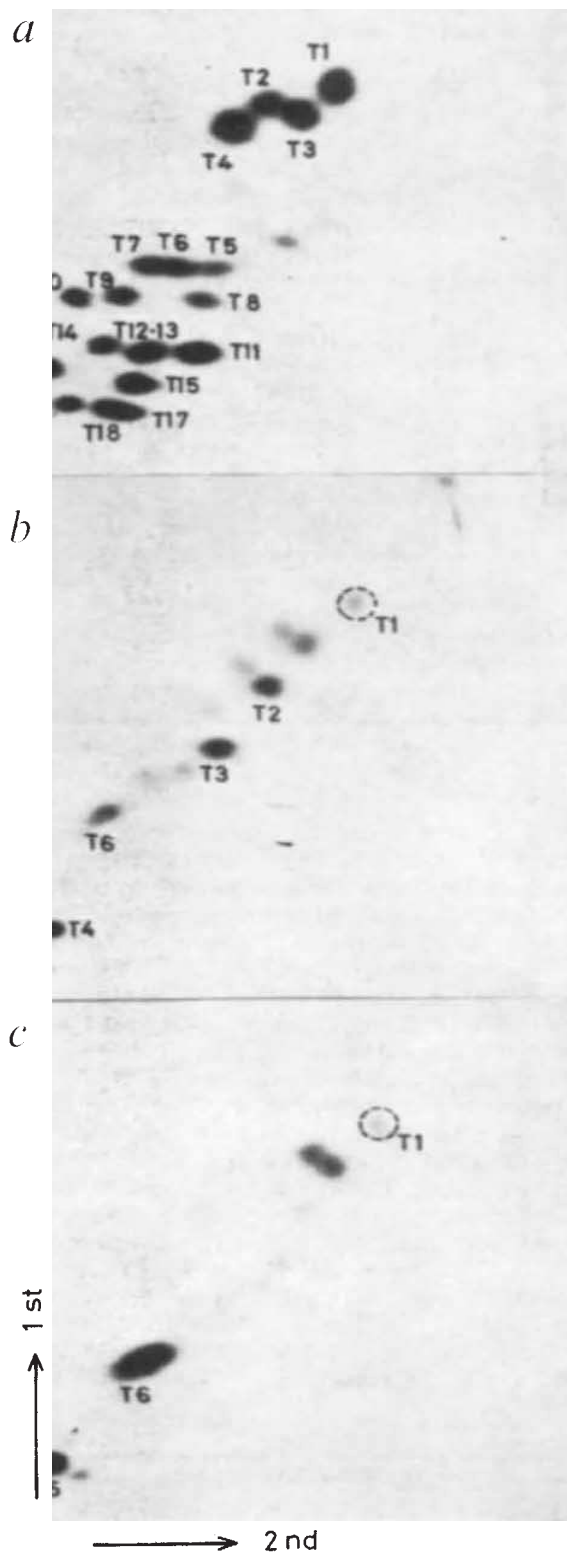


Fig. 3 Two-dimensional fingerprints of T₁ RNase digests of (a) RNA synthesised on Hap-Hga V, and (b), (c) (A)₃ U-primed RNA formed on the RNA polymerase binding site. (a) (*G) RNA was synthesised on Hap-Hga V as in the legend to Fig. 2c and digested with T₁ RNase. The digest was applied to a 20 cm × 20 cm PEI-cellulose plate and chromatographed as in ref. 6. The sequences of the spots numbered are given in Table 1. (b and c) (*G) RNA was synthesised on the heat-denatured RNA polymerase binding site and fractionated as in the legend to Fig. 2f. Bands 1 and 2 indicated in Fig. 2f were digested with T₁ RNase and chromatographed as in (a). (b) Fingerprint of Band 2. (c) Fingerprint of Band 1. The sequences of spots numbered are given in Table 2.

Table 1 Analysis of nucleotides in pancreatic and T₁ RNase digests of RNA formed on Hap-Hga V

Spot no.	T ₁ RNase products Sequence*	Molar ratio	Spot no.	Pancreatic RNase products Sequence*	Molar ratio
T1a	G <u>A</u>	2	P1a	C <u>A</u>	3
T1b	G <u>G</u>	2	P1b	C <u>C</u>	2
T1c	G <u>U</u>	1	P1c	C <u>G</u>	2
T2	CG <u>G</u>	1	P1d	C <u>U</u>	4
T3	AG <u>G</u>	1	P2a	GC <u>A</u>	1
T4a	CAG <u>U</u>	1	P2b	GC <u>G</u>	1
T4b	ACG <u>C</u>	1	P2c	GC <u>U</u>	1
T4c	ACG <u>A</u>	1	P3	GAC <u>G</u>	1
T5	UAAAG <u>A</u>	1	P4	GAAC <u>U</u>	1
T6	AACUG <u>U</u>	1	P5	AAAGC <u>A</u>	1
T7	ACCU <u>A</u>	1	P6	AAAGAC <u>C</u>	1
T8	UCUG <u>C</u>	1	P7	GGAC <u>G</u>	1
T9	AUCCG <u>C</u>	1	P8a	U <u>A</u>	4
T10	CUAUCCAG <u>U</u>	1	P8b	U <u>C</u>	5
T11	UAUUG <u>G</u>	1	P8c	U <u>G</u>	7
T12	CAUUUG <u>A</u>	1	P8d	U <u>U</u>	9
T13	UUUAAAG <u>C</u>	1	P9a	AU <u>C</u>	1
T14	AUUCAAUG <u>A</u>	1	P9b	AU <u>G</u>	2
T15	AUUUAUG <u>G</u>	1	P9c	AU <u>U</u>	4
T16	UCAUUCUG <u>U</u>	1	P10	AAU <u>G</u>	1
T17	AUUUUUG <u>A</u>	1	P11	GU <u>U</u>	2
T18	UUUUCUG <u>A</u>	1	P12a	GAU <u>U</u>	3
T19	AAUAUUUAUG <u>A</u>	1	P12b	AGU <u>A</u>	1
T20	pppG <u>U</u>	1	P12c	AGU <u>C</u>	1
			P13	GAAU <u>A</u>	1
			P14	GGU <u>C</u>	1
			P15	GAGGGGAU <u>U</u>	1
			P16	pppGU <u>A</u>	1
			P17	GGAAU <u>C</u>	0.5

* Nucleotide linked to the 5'-end is underlined.

procedure were separated by sucrose density-gradient centrifugation. On adding Hap-Hga V to an RNA synthesising mixture, a uniform RNA which migrated near 5S ribosomal RNA as marker was efficiently synthesised (Fig. 2c). The RNA only hybridised to the (–) strand of the template, like those made on intact RF-I DNA. For isolation of the RNA polymerase binding site, Hap-Hga V was mixed with RNA polymerase and GTP, digested with DNase I and the portion protected was isolated as in the legend to Fig. 2e. The gel electrophoretic pattern of the polymerase binding site prepared by this procedure is shown in Fig. 2e. The average chain length estimated from the analysis of the terminal P was roughly 45. To synthesise RNA on this fragment, the fragment was denatured by heating, and incubated in an RNA synthesising mixture containing a primer oligonucleotide. The synthesised RNA was isolated, and fractionated by hybridisation to fd single-stranded (SS) RNA [(+) strand]. The RNA fraction complementary to fd SS DNA was collected, and further resolved by gel electrophoresis (Fig. 2f). The detailed conditions for RNA synthesis and the procedure for fractionation of RNA are given in the legend to Fig. 2f. Hydrolysis of RNA with RNases and analysis

of the digests by two-dimensional chromatography on PEI (polyethylenimine)–cellulose plates were as described previously⁶.

Sequence of RNA synthesised

RNA was synthesised on Hap-Hga V with each of the four nucleoside α³²P-triphosphates (abbreviated as (*N)RNA, where N = A, C, G, or U), and hydrolysed with either pancreatic RNase or T₁ RNase. The resulting nucleotides were resolved on PEI–cellulose plates. A typical fingerprint obtained from (*G)RNA is shown in Fig. 3a. The distribution of ³²P in each spot was determined. In addition, the nearest neighbour analysis was carried out on each spot after the complete digestion with T₂ RNase. All the nucleotides obtained and their approximate molar ratios are summarised in Table 1.

The sequence of shorter oligonucleotides was determined simply by the distribution of ³²P in nucleotides. For analysis of longer oligonucleotides, each digestion product was further cleaved using either pancreatic RNase or T₁ RNase, and chromatographed. The products were digested with T₂ RNase, and ³²P in nucleotides determined. The sequences for all the

Table 2 Analysis of nucleotides in pancreatic and T₁ RNase digests of (A)₃ U-primed RNA formed on the RNA polymerase binding site

Spot no.	T ₁ RNase products Sequence †	Molar ratio	Spot no.	Pancreatic RNase products* Sequence†	Molar ratio
T1	G <u>U</u>	1	P1	C <u>C</u>	1
T2	AAG <u>C</u>	0.8	P2	AC <u>C</u>	1
T3	UCAG <u>A</u>	1	P3	AAAU <u>C</u>	1
T4	UCUAUUUAUG <u>U</u>	1	P4	AAAAAU <u>C</u>	0.4
T5†	U(CUU)UACCCUG <u>U</u>	1	P5	GU <u>C</u>	1
T6	AAAUCAG <u>G</u>	0.6	P6	AGU <u>C</u>	1
T7	AAAUCAAAAUCAG <u>G</u>	0.4	P7	AGGU <u>C</u>	1
			P8	AGAAGC <u>A</u>	0.7

* The products obtained from (*C)RNA are listed.

† Nucleotide linked to the 5'-end is underlined.

‡ The position of C in parentheses is not determined by the nearest neighbour analysis.

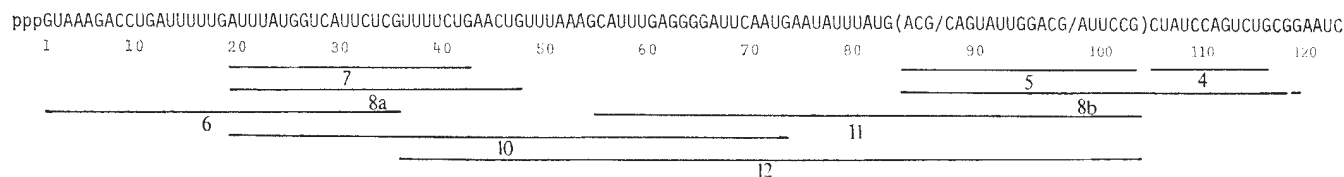


Fig. 4 The nucleotide sequence of RNA synthesised on Hap-Hga V. The region covered by each partial T_1 RNase product is underlined. The numbers represent the bands indicated by the side of Fig. 2d. Each band has been repurified using gel electrophoresis in urea.

oligonucleotides were determined in this way, except those for spots T10, T16, T18 and T19 in the T_1 RNase digest. The nearest-neighbour analysis of T10 deduced four possible sequences, CAUCCUAG, CCUAUCAG, CCAUCUAG, and CUAUCCAG. As the nucleotide linked to the 3'-end is G, we looked for three possible sequences, GCA, GCC and GCU, in the pancreatic RNase digest. Table 1 shows only one GCU in P2c and two GCA in P2a and P5. As two GCA appear in T4a and T12 in the T_1 RNase digest, we concluded that the sequence for T10 is CUAUCCAG. The analysis of T16 deduced two possible sequences, UCAUUCUG and UCUCAUUCG. The sequence was determined to be UCAUUCUG by digestion with U_2 RNase. Two alternative sequences were also obtained for T19, AAUAUUUAUG and AUUUAUAUG. Two possible sequences, GAAUA and GAUU, were looked for in the pancreatic RNase digest. There are three GAUU in P12a and one GAAUA in P13. The three GAUU appear in T9, T14 and T15 in the T_1 RNase digest. Thus the sequence for T19 was deduced to be AAUAUUUAUG. The nearest-neighbour analysis did not provide information on the position of C in the U cluster of T18. This sequence has been determined to be UUUUCUG in a separate experiment, in which the sequence of RNA formed on the (+) strand of Hap-Hga V in the oligonucleotide primed reaction was analysed. The sequences of most oligonucleotides in Table 1 have also been confirmed by this type of analysis.

To reconstruct RNA from the T_1 RNase products, partial T_1 RNase digests were prepared and resolved by gel electrophoresis (Fig. 2d). The resulting bands were redigested with

reduced. RNA synthesis was stimulated approximately three-fold by adding $(A)_3U$ as primer. We chose this oligonucleotide as primer because RNA formed on Hap-Hga V contained the sequences, AUUU and AUUUUU, near the starting end (see Fig. 4).

RNA was synthesised with each of the four nucleoside $\alpha^{32}P$ -triphosphates, fractionated by hybridisation to fd SS DNA, and resolved by gel electrophoresis. Note that the direction of RNA thus prepared is the reverse of the RNA made on 'intact' Hap-Hga V. The major band obtained (Band 2 in Fig. 2f) was digested with T_1 RNase and chromatographed on PEI-cellulose plates. A typical fingerprint obtained from $(^*G)RNA$ is shown in Fig. 3b. The major spots detected are listed in Table 2. The amounts of spots T1 to T5 were about equimolar. The sequence of each spot was analysed as in the previous section. For analysis of longer oligonucleotides, digestion was carried out using either pancreatic RNase or U_2 RNase. Unique sequences were obtained for all spots, except for T5. The sequence deduced for T5 was U(CUU)UACCCUG, in which the position of C in parentheses was not determined. Spots T6 and T7 contained AAAU at the 5'-end and overlapped with the sequence at the 3'-end. These sequences are just complementary to the sequence which either positions 9 to 15 or positions 9 to 22 on the RNA made on Hap-Hga V (see Fig. 4). In addition, the sum of T6 and T7 was about equimolar with each other spot (see Table 2). Thus, we concluded that the leftward RNA analysed had been initiated with primer at either position 15 or position 22 on the sequence in Fig. 4. On the basis of analysis on nucleotides adjacent to the terminal G, all the

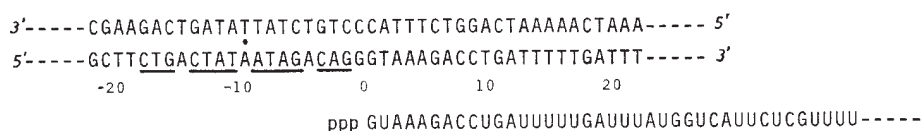


Fig. 5 The nucleotide sequence of the RNA polymerase binding site on Hap-Hga V. The starting sequence of RNA formed on Hap-Hga V is indicated for comparison. The region with twofold symmetry is underlined. The axis of symmetry is at the -10 position.

T_1 RNase and chromatographed on PEI-cellulose plates. On the basis of the result obtained and information on the nucleotide adjacent to the terminal G of each spot, all the T_1 RNase products were ordered into a sequence of RNA, except those for the region between the positions 84 and 104 (Fig. 4). The arrangement of three segments in parentheses is not determined yet. The yield of P17(GGAAU) which was assigned to the 3'-end of the RNA was less than one mole in the pancreatic RNase digest (see Table 1). This suggests that the RNA chains were randomly terminated after position 120.

Sequence of RNA polymerase binding site

As shown in Fig. 2e, the fraction of Hap-Hga V which was protected from nucleolytic digestion by the polymerase binding gave a relatively uniform band on gel electrophoresis. To analyse the sequence of such a region, the DNA fragment isolated was denatured by heating and added to an RNA synthesising mixture in which the substrate concentration was

T_1 RNase products obtained can be ordered either (1) T6(T7): T1 : T4 : T5 : T3 : T2 or (2) T6(T7) : T1 : T5 : T4 : T3 : T2. The combination was determined as (2) using the analysis of Band 1 in Fig. 2f. Band 1 is assumed to be an intermediate, as this band was shifted to the Band 2 region by extending the incubation period. On chromatography of the T_1 RNase digest of Band 1, the major spots detected were only T1, T5 and T6. In Fig. 3c, a fingerprint obtained from $(^*G)RNA$ is shown. Note that spot T1 is only obtained from $(^*U)RNA$. In other experiments, T7 was also detected in addition to these three spots. According to this combination, the sequence subsequent to T6(T7) becomes complementary to the starting sequence of RNA made on Hap-Hga V.

In Fig. 5, the sequence of RNA finally deduced is shown as the sequence of DNA. The sequence has been confirmed by the analysis of the pancreatic RNase products (Table 2). The sequence would represent only the major part of the polymerase binding site. Nevertheless, the size of the sequence determined was about equal to the average size of the DNA fragment isolated as the polymerase binding site. Figure 5 shows that the

right half of the sequence is just identical with the starting sequence of RNA initiated on Hap-Hga V, indicating that transcription is initiated in the centre of the binding site. The other feature of the sequence is that a region with twofold symmetry is contained in the non-transcribed part, as indicated in Fig. 5. Such symmetrical regions have been detected in the polymerase binding site of other promoters^{8,9}, although each sequence is different. Initiation of transcription would include at least the processes of recognition of the promoter signal and formation of an initiation complex by the polymerase. If these processes occur at different parts of a promoter, the polymerase binding site we analysed would be a part of the promoter. As mentioned earlier, RNA chains are efficiently initiated on Hap-Hga V, indicating that this fragment contains all of the sequence information needed for the promoter function. The length of the non-transcribed region of this fragment is about 100 base pairs. Sequencing of such a region will provide more information about the feature of the promoter.

In the course of this study, we learned that Schaller, Gray and

Herrmann¹⁰ have determined the sequence of a strong RNA polymerase binding site on fd by direct DNA sequencing. The sequence obtained by these authors coincides with the sequence of the polymerase binding site presented in this paper. They also note a region with strong symmetry in the non-transcribed part of the binding site.

We thank Dr. Schaller for the exchange of unpublished information.

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¹ Takanami, M., Okamoto, T., and Sugiura, M., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 179-187 (1970).

² Heyden, B., Nusslein, Ch., and Schaller, H., *Nature new Biol.*, **240**, 9-12 (1972).

³ Okamoto, T., Sugiura, M., and Takanami, M., *Nature new Biol.*, **237**, 108-109 (1972).

⁴ Gilbert, W., and Maxam, A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3581-3584 (1973).

⁵ Takanami, M., *FEBS Lett.*, **34**, 318-322 (1973).

⁶ Mirzabekov, A. D., and Griffin, B. E., *J. molec. Biol.*, **72**, 633-643 (1972).

⁷ Peacock, A. C., and Dingman, C. W., *Biochemistry*, **6**, 1818-1827 (1967).

⁸ Maniatis, T., Tashne, M., Barrell, B. G., and Donelson, J., *Nature*, **250**, 394-397 (1974).

⁹ Sekiya, T., and Khorana, H. G., *Proc. Natn. Acad. Sci. U.S.A.*, **71**, 2978-2982 (1974).

¹⁰ Schaller, H., Gray, C., and Herrmann, K., *Proc. natn. Acad. Sci. U.S.A.* (in the press).

letters to nature

Radiation as a source of gravitation

I HAVE investigated a few steady state cases according to general relativity, and some of the results are of interest.

Throughout this letter, relativistic units ($c = 1$, $G = 1$), and ray optics are used.

(1) Spherical symmetry, purely radial rays. The metric is

$$ds^2 = e^{2v} dt^2 - (1 - 2\Phi)^{-1} dr^2 - r^2(d\theta^2 + \sin^2\theta d\psi^2) \\ v = v(r), \quad \Phi = \Phi(r) \quad (1)$$

Imposing $T = T_2^2 = T_3^3 = 0$ and defining

$$4\pi r^2 T_0^0 = -4\pi r^2 T_1^1 = \varepsilon(\Phi)$$

the equations reduce to

$$\frac{d\varepsilon}{d\Phi} = \frac{2\varepsilon}{1-2\Phi} \frac{\Phi+\varepsilon}{\Phi-\varepsilon} \frac{1}{r} \frac{dr}{d\Phi} = -\frac{1}{\Phi-\varepsilon}, \quad \varepsilon e^{2v} = \text{constant} \quad (2)$$

Note that ε , a pure number, is the sum of the equal inward and outward fluxes of radiation, and that, for comparison, the

outward flux of the Sun is $\varepsilon = 10^{-26}$, while a quasar might perhaps reach 10^{-4} .

The solutions of equation (2), in the relevant region ($\Phi < \frac{1}{2}$, $\varepsilon \geq 0$) can be expressed in closed form in terms of $x = (\frac{1}{2} + \varepsilon)[\varepsilon(1-2\Phi)]^{-1/2}$. They start at the origin along $\varepsilon = \Phi$ (Fig. 1) (region A) then sharply slice off towards the right, proceed towards higher values of Φ with an only imperceptibly increasing ε (assumed to be fairly small) (region B) until, shortly before $\Phi = \frac{1}{2}$ is reached, ε begins to grow rapidly, with Φ soon passing a maximum and then diminishing (region C), while ε still grows. The line $\Phi = 0$ is crossed, and ε grows until $\varepsilon = -\Phi$ whence (region D) $\varepsilon > 0$ as $\Phi \rightarrow -\infty$. Note that the origin corresponds to $r = \infty$, that the radius drops very sharply along the curve in region A, quite fast in B, but only gently as we proceed through C and D with $r \rightarrow 0$ as $\Phi \rightarrow -\infty$.

It is immediately plain that our normal picture of such a system is represented by A and the adjacent parts of B, and that C and D are the unexpectedly peculiar neighbourhood of the centre where focusing produces a curious singularity, probably irreproducible in practice owing to the non-zero wavelength of light.

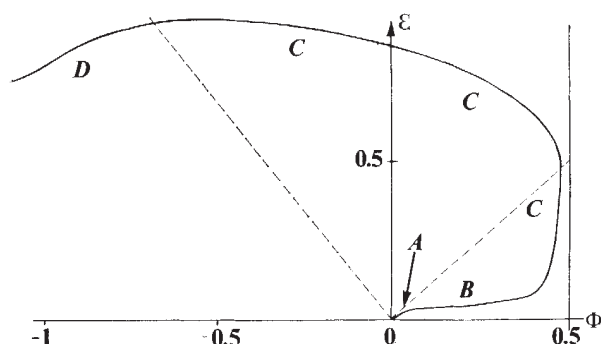
To avoid the centre, radiation may be limited to $r \geq r_1$ by putting a material body in. Most simply, if at $r = r_1$ there is a thin material shell with positive T and T_0^0 and empty interior, then necessarily

$$0 < \Phi_1 < 4/9, \quad \varepsilon_1 < \frac{1}{2}[1 - (1 - 2\Phi_1)^{1/2}][3(1 - 2\Phi_1)^{1/2} - 1] \quad (3)$$

so that we are confined to regions A and B. The zone of radiation may also be terminated by an outer shell, with empty space beyond. Though the conditions are a little more complex than at an inner shell, in fact the radiation pressure helps to support the shell.

Without an internal body, the central conditions do not select any particular solution of equation (2). Indeed the central singularity is such that on this ray picture it should be possible to confine, by an external shell, the zone of radiation to C and D, and even to terminate it with Φ so negative that, even with the necessarily positive mass of the shell, the external solution is one of negative mass.

Fig. 1 Schematic diagram of a typical solution of equation (2).



(2) As a second example, one could treat this case in a quasi-Newtonian fashion by taking the flux ϵ to be constant. Therefore the energy content and thus supposedly the mass would depend linearly on the radius, being proportional to it if the radiation extends to the centre. In this last case the potential Φ would equal ϵ and be constant throughout (corresponding to region *A* above), in the more general case of a central body of mass exceeding that of its volume filled with appropriate radiation $\Phi - \epsilon$ would be a positive multiple of r^{-1} (corresponding to region *B* above), whereas there seems to be no relativistic case corresponding to a central body with mass less than that of its volume filled with the continuation of the radiation.

Note that metric (1), with $\Phi = \text{constant}$, $rdv/dr = \text{constant}$, leads to a non-trivial constant energy tensor T_{μ}^{μ} which, for small equal and opposite values of the constants, gives radial radiation in the first approximation.

(3) Cases of cylindrical symmetry. These may be static or stationary depending on whether the fluxes parallel to the positive and negative z directions are everywhere equal or not.

Consider now a static case characterised by an amplitude A and apply a Lorentz transformation of velocity V parallel to the z axis. The flux against V will be enhanced and that in the opposite direction diminished. Now let $V \rightarrow 1$, while letting $A \rightarrow 0$ suitably. In the limit one arrives at a system with a unidirectional flow of energy. Unidirectional cases so derived form a special class (which of course could have been derived directly) with the property that all rays are parallel to the axis. The existence of this class is somewhat surprising since after all the stream of energy near the axis should act as a source of gravitation leading to rays weaving in and out, crossing each other along the axis of symmetry and being pulled back from further out by the gravitational force, as is indeed the case in general for cylindrically symmetrical systems.

With the appropriate general metric (all coefficients are functions of r only)

$$ds^2 = e^{2\sigma} [e^{2\lambda} \cos 2\gamma dt^2 + 2 \sin 2\gamma dt dz - e^{-2\lambda} \cos 2\gamma dz^2] - e^{2\delta} dr^2 - r^2 d\psi^2 \quad (4)$$

this particular class is given by

$$\delta = \sigma = 0, \quad w(\gamma) \equiv r d\gamma/dr = \pm r \cos 2\gamma d\lambda/dr$$

giving

$$8\pi T^{00} = \frac{e^{-2\lambda}}{2r^2(\pm 1 - \sin 2\gamma)} \frac{d}{d\gamma} (w^2 \cos^2 2\gamma)$$

naturally with the trace of the energy tensor and its radial and transverse components vanishing.

(4) More generally, acceptable solutions of equation (4) representing pure radiation without transverse (ψ) components are described by equation (4) in the notation $u = rd\sigma/dr$, $v = rd\lambda/dr$, $w = r d\gamma/dr$ by

$$\delta = 2\sigma,$$

$$2rdu/dr = 2u + u^2 - v^2 \cos 2\gamma + w^2 \geq 0,$$

$$rdv/dr \cos 2\gamma \geq 4vw \sin 2\gamma,$$

$$(rdv/dr \cos 2\gamma - 4vw \sin 2\gamma)^2 \geq (rdu/dr)^2 + (rdw/dr - 2v^2 \sin 2\gamma \cos 2\gamma)^2$$

equality in the last case implying unidirectional flow of energy. Full details will be published elsewhere.

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Spectra of the Jupiter radio bursts

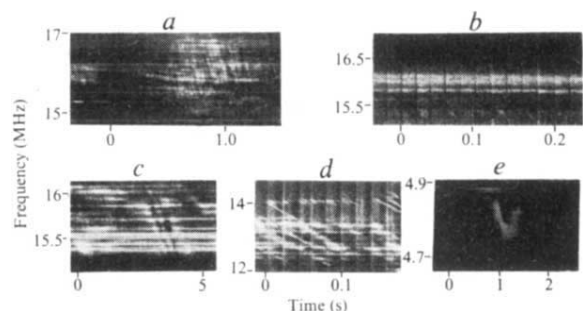
BETWEEN March and September of 1973 and 1974, observations were made of the Jupiter radio bursts with the Llanherne filled-aperture low frequency radio telescope¹ (650 m $\times$ 650 m). The signals over the frequency range 4–29 MHz were recorded directly on to six video tape recorders for 5 min each day near transit. Some observations at 15–17 MHz were also obtained between May and September 1972. Frequency-time spectra were subsequently made with a 256-channel real-time analyser with channels 10 kHz wide and separated by 10 kHz. The spectra were recorded on 35-mm film moving at a variety of speeds from 150 inch min⁻¹ to 3 inch min⁻¹. When records with higher frequency resolution (2 kHz) or time resolution (100 μ s) were required, a time expansion spectrum analyser² with 1,500 channels in a 3 MHz bandwidth was used. Over the observing period, tape records of the Jupiter radio bursts were obtained during about two-thirds of all transits, and so far about 80 h of video tape containing the bursts has been accumulated. Here I present a preliminary description of the chief properties of their spectra.

Like the solar radio bursts, the dynamic spectra provide a convenient basis for classification, and the existence of at least two classes, the L bursts with a time scale of seconds and the S bursts with a time scale of milliseconds, has been recognised by earlier observers^{3,4}. But with the better time and frequency resolution now available it has become clear that there is considerably more variety in the radio phenomena as well as much more fine structure.

The L or lane bursts are seen on the frequency-time ($f-t$) plane as a diffuse background crossed by drifting modulation lanes (Fig. 1*a*). The ($f-t$) slope is ~ 0.2 MHz s⁻¹. Riihimaa³ has identified several subclasses of L bursts on the basis of the sign of df/dt , which may be positive or negative or both. The slope is a function of the system III longitude of Jupiter and the modulation is therefore likely to be associated with the emission and propagation of the radiation near the planet. The lanes are quasi-periodic in time with a period of about 3 s near 21 MHz and are observed from 8 to 30 MHz (ref. 3).

Other types of lane structure have been seen in the present observations. When the time scale is expanded by a factor of 10, a second class, the fast lane (FL) bursts, is observed in which the ($f-t$) slope of modulation is near 2.0 MHz s⁻¹ (Fig. 1*a*). Again, both positive and negative $f-t$ slopes are observed. The quasi-period of the modulation is approximately 30 ms. FL bursts were observed between 5 and 17 MHz on 29 out of 56 occasions on which Jupiter bursts were recorded between April 22, 1973, and July 2, 1973. Within this frequency range they have the unusual property that the $f-t$ slope of the modulation lanes is independent of frequency (Fig. 2). At still higher time resolution a third class, the very fast lane (VFL) bursts, was observed on a few occasions both

Fig. 1 Dynamic spectra of Jupiter radio bursts. *a*, Lane bursts and fast lane bursts; *b*, very fast lane bursts; *c*, shadow drift pair burst; *d*, S bursts; *e*, U burst.



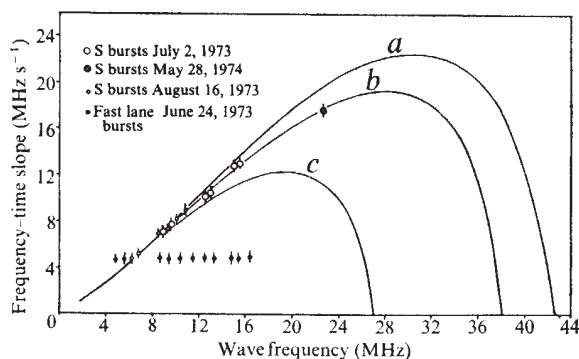


Fig. 2 Observed variation of df/dt with wave frequency for S bursts and fast lane bursts. Solid lines show the theoretical variation for cyclotron radiation from electrons of speed $0.1c$ travelling in the Io L shell for different values of equatorial pitch angle ϕ_0 . Magnetic field dipole moment $4G R_J^3$. a, $\phi_0 = 2^\circ$; b, $\phi_0 = 2.16^\circ$; c, $\phi_0 = 2.5^\circ$.

in the 14–17 MHz and 4–7 MHz frequency ranges (Fig. 1b). The f - t slope is approximately 15 MHz s^{-1} and is again independent of frequency between 16 and 4 MHz s^{-1} . The quasi-period of the modulation lanes for these bursts is near 4 ms.

A separate class of bursts, the shadow drift pairs, is illustrated in Fig. 1c. A typical spectrogram shows a pair of parallel dark bands with a mean separation in time of 0.4 s and a mean f - t slope of 1.4 MHz s^{-1} . They bear a remarkable resemblance to the solar radio drift pair bursts, except that they are seen in absorption rather than emission. Like these latter bursts, their properties vary only over a small range. Ten examples were recorded in July and August 1972.

Short, or S, bursts with a single frequency duration of 1 to 10 ms were observed during about half the Jupiter radio events between April and July 1973 (Fig. 1d). Their f - t slope is 10 MHz s^{-1} at 12 MHz, and is very nearly proportional to the wave frequency over the frequency range 5 to 15 MHz (Fig. 2). Their instantaneous bandwidth is typically 10 kHz, although it may be as small as 3 kHz. These measured values of df/dt are likely to be too high by approximately 0.008 MHz s^{-1} if account is taken of the effect of the analyser bandwidth⁵ on the variable frequency signal. Within the millisecond time scale of the S bursts, there are many fine structure phenomena which resemble those seen in the terrestrial v.l.f. emissions. Triggered and periodic bursts occur frequently, and there is much evidence of wave-particle interactions from sudden changes in f - t slope where S burst spectra cross constant frequency emissions. On a few occasions, S bursts with reverse (positive) f - t slope are observed. A rare variant of the S bursts is the U burst, or inverted U burst, which shows a quasi-parabolic change and reversal in f - t slope (Fig. 1e). U bursts have been seen so far only below 10 MHz. Like the shadow drift pairs, the f - t spectra of the U bursts resemble those of the corresponding solar radio emissions, the solar U bursts.

The distribution of f - t slopes of all these bursts is shown in Fig. 3, where three main regions near 0.1 MHz s^{-1} , 1.0 MHz s^{-1} and 10 MHz s^{-1} may be seen. Similar groupings of df/dt exist for the solar radio emissions. So far no satisfactory explanation of the lane bursts has been advanced, although it has been suggested by Riihimaa³ that the modulation results from interference between waves from multiple sources. The invariance of df/dt with f seems difficult to account for by this process. Riihimaa also proposed, alternatively, that the lane structure is caused by the passage of the radiation through a moving spatially periodic screen after emission. Since different frequencies would be generated at different points along a field line, the frequency interval between the modulation lanes would be determined by the spatial period.

The properties of the S bursts, on the other hand, were predicted from a consideration of the likely radio emission from moving sources travelling outwards along the Jupiter magnetic field lines and radiating in the doppler shifted cyclotron mode⁶. As in the Earth's magnetosphere, the sources may be convective instabilities in almost monoenergetic electron streams⁷ or possibly isolated bunches of electrons as in the solar corona. The f - t slope is then a measure of the speed v of the source, and for a simple dipole field

$$df/dt \propto f^{4/3} A(\lambda, \lambda_m) v$$

$A(\lambda, \lambda_m)$ is zero for magnetic latitude $\lambda = 0$ or λ_m , the mirror latitude. If the magnetic dipole moment and the L shell of the electrons can be assumed, then their velocity and equatorial pitch angle ϕ_0 can be obtained from observations of df/dt over a sufficiently wide range of f . Figure 3 compares the observations with the calculated variation of df/dt with f for $v = 0.1c$ and $\phi_0 = 2^\circ, 2.16^\circ$, and 2.5° respectively for electrons in the L shell of the satellite Io (Jupiter dipole moment assumed⁸ to be $4 \text{ gauss } R_J^3$). The mirror-point cyclotron frequency of the electrons is 38 MHz. It should be noted that if the maximum magnetic field cyclotron frequency fH_m near the planet's surface proves to be less than this, then this result may imply that the electrons are accelerated near the surface⁹. Two alternative revised models of the magnetic field⁸ derived from Pioneer 10 observations give $fH_m = 31$ and 38 MHz respectively. But fH_m is very strongly dependent on the precise location of the dipole centre and further revisions can be expected in the future.

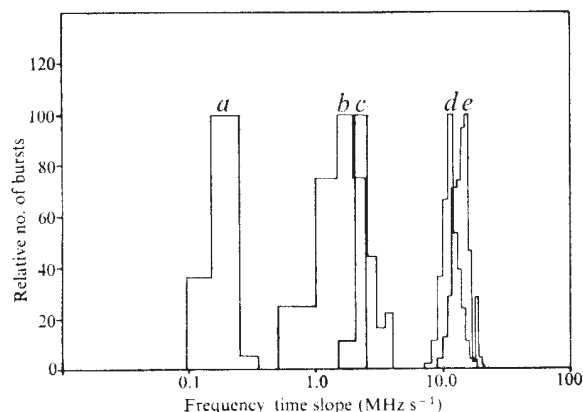


Fig. 3 Distribution of frequency-time slopes of: a, lane bursts; b, shadow drift pairs; c, fast lane bursts; d, S bursts; e, very fast lane bursts. Frequency range 15–17 MHz.

The S bursts do not normally extend in frequency over more than 1–2 MHz and do not individually exhibit the variation of df/dt with f shown in Fig. 1d. Indeed, the f - t trace may be curved in either direction, not just linear. The relatively small frequency range would be expected if the radiation is from sources travelling along curved field lines and strongly beamed as expected for cyclotron radiation. Slight variations in the speed of convective beam instabilities would account for the f - t deviations. The very narrow spread in the distribution of df/dt for observations made at different times is particularly striking and contrasts, for example, with the wide distribution for type III solar bursts. It points to a source of radiating electrons of almost constant energy such as has been suggested for the model of Goldreich and Lynden-Bell¹⁰, where the satellite Io acts as a unipolar inductor and a source of constant e.m.f. across the Io flux tube as it moves through the Jupiter magnetic field.

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- ¹ Ellis, G. R. A., *Proc. astr. Soc. Aust.*, **2**, 135 (1972).
- ² Ellis, G. R. A., *Aust. J. Phys.*, **26**, 253 (1973).
- ³ Riihimäki, J. J., *Report 522* (Univ. Oulu, Finland, 1971).
- ⁴ Gallet, R. M., in *Planets and Satellites* (edit. by Kuiper, G., and Middlehurst, B. M.), chapter 14 (Univ. Chicago Press, Chicago, 1961).
- ⁵ Storey, L. R. O., and Grierson, J. K., *Electronic Eng.*, **30**, 648 (1958).
- ⁶ Ellis, G. R. A., *Radio Sci.*, **69D**, 1513 (1965).
- ⁷ Helliwell, R. A., *Whistlers and Related Ionospheric Phenomena* (Stanford University Press, Stanford, California, 1965).
- ⁸ Smith, E. J., Davis, L., Jr, Jones, D. E., Coleman, P. J., Jr, Colburn, D. S., Dyal, P., Sonett, C. P., and Frandsen, A. M. A., *J. geophys. Res.*, **79**, 3501 (1974).
- ⁹ Piddington, J. H., *Cosmic Electrodynamics*, **3**, 240 (1972).
- ¹⁰ Goldreich, P., and Lynden-Bell, D., *Astrophys. J.*, **156**, 59 (1969).

Alternative hypothesis for the origin of CCF xenon

I SUGGEST that the anomalous xenon isotopic composition known as carbonaceous chondrite fission (CCF) xenon is not caused by fission, but is the direct result of a modified r-process nucleosynthesis which produces an abundance peak at $Z = 54$ and the magic neutron number $N = 82$. I further propose that the xenon so produced ('R xenon') was trapped in dust grains, which were subsequently incorporated in the solar system with minimal degassing. This hypothesis also provides a natural explanation for the intimate association between R xenon and a newly discovered xenon component in primitive meteorites^{1,2}.

Analyses of the abundance and isotopic composition of xenon in meteorites have provided insight into several aspects of the early solar system and the origin of some of the complex patterns are now well understood. Recently, for example, it was confirmed that xenon in Ca-rich achondrites arises, in part, from the spontaneous fission of extinct ²⁴⁴Pu (ref. 3).

One of the more enigmatic xenon compositions is found in carbonaceous meteorites and unequilibrated ordinary chondrites⁴, both of which are thought to be primitive meteorite types. It has properties which are characteristic of xenon produced from fissioning nuclides, hence its name, the CCF component.

It is still not clear whether this component actually has a fission origin, at least to the extent that the parent nuclide has not been identified. In order to identify the parent nucleus, the isotopic composition of the meteoritic xenon component must be compared with known fission spectra. Lack of identification could once have been attributed to a paucity of experimental data on fission spectra from nuclei in the mass range $240 \leq A \leq 260$, but this is not now the case⁵. A comparison of all presently known fission spectra with the meteoritic composition does not, however, yield any match (D.C.B., unpublished).

Failure to identify the hypothetical parent of the xenon component has not given rise to alternative interpretations but, rather, has generated more exotic suggestions as to the parent⁶.

Anders and Heymann⁶, and more recently Takaoka⁸, have shown that the amount of R xenon in a meteorite correlates with the abundance of trapped ¹³²Xe. This correlation is generally interpreted as a statement that the 'parent' nuclide which fissioned to give rise to R xenon was a volatile element⁶. There are, however, other interpretations, which I shall discuss later. The calculated absolute abundance of R xenon depends on the assumed trapped xenon isotopic composition, but is generally characterised by ¹³⁶Xe concentrations of $\sim 0.1 \times 10^{-10}$ to 1.0×10^{-10} cm³ STP g⁻¹.

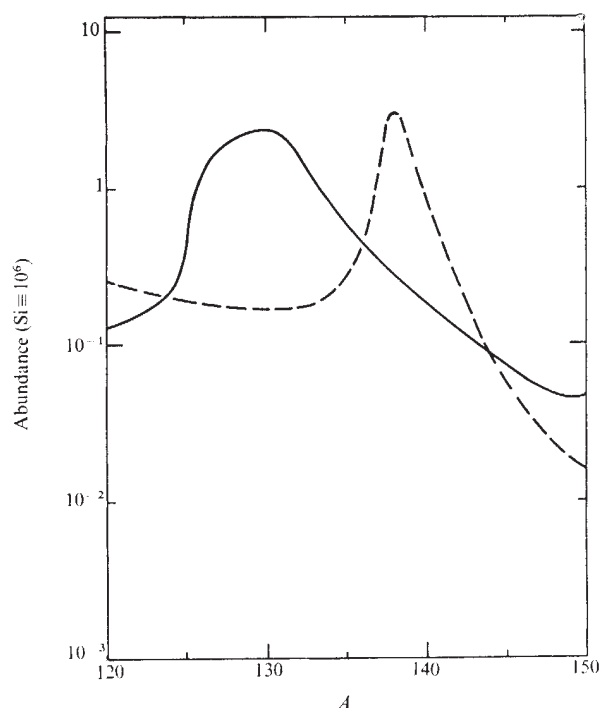


Fig. 1 Schematic representation of the abundance curves expected from 'classical' r-process (—) and s-process (---) nucleosynthesis in the mass range $120 \leq A \leq 150$. Data from ref. 20.

The most recent estimate (ref. 9 and R. O. Pepin, private communication) of R xenon is given in Table 1. This estimate differs from previous ones^{10,11} primarily in that the ¹³²Xe/¹³⁶Xe ratio is lower (0.22 compared with 0.37) but is within the uncertainties associated with the earlier estimates. The new estimate agrees with that given by Marti¹² for ¹³⁶Xe:¹³⁴Xe:¹³²Xe.

As pointed out by Phinney¹ and Manuel *et al.*², analyses of xenon isotopic compositions evolved from carbonaceous meteorites during stepwise heating experiments reveal the existence of an isotopically distinct xenon component which is characterised by a progressive enrichment (¹²⁴Xe > ¹²⁶Xe) in the isotopes of xenon which are shielded from contributions by way of fission. These workers have shown that the new component ('P xenon') does not arise from *in situ* nuclear processes and that it seems to be fairly uniformly mixed with R xenon. Manuel *et al.* designate the mixture of P xenon and R xenon as Component X. More recent studies¹³ indicate that P xenon may be present in most primitive meteorites. Manuel and his co-workers do not speculate in detail as to the origin of P xenon, but they do suggest that P xenon and R xenon 'result from a common source'. In this regard, it is worth noting that Phinney attributes P xenon to p-process nucleosynthesis.

There is increasing evidence that some of the particulate matter (dust grains) in the primitive solar nebula were not heated sufficiently to degas quantitatively or chemically equilibrate with the bulk of the nebula, and have retained in part the elemental and isotopic characteristics of a period which predates the formation of the solar system. I shall designate such isotopic compositions as 'non-solar'. The first evidence was the discovery of a singular neon isotopic composition in carbonaceous meteorites¹⁴, characterised by a trapped ²⁰Ne/²²Ne ratio ≤ 3.4 , which was suggested to be 'non-solar'¹⁵. Subsequently, analyses of oxygen isotopic ratios in carbonaceous meteorites have led to the discovery of an isotopic anomaly which has also been described as 'non-solar'¹⁶. Work by Gray and Compston¹⁷ and Lee and Papanastassiou¹⁸ has revealed the

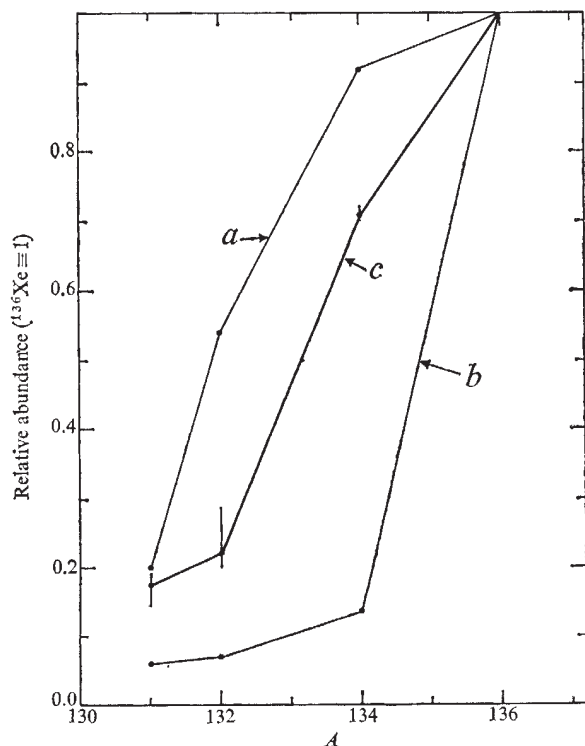
Table 1 Isotopic composition of R xenon

^{131}Xe	^{132}Xe	^{134}Xe	^{136}Xe	Ref.
—	0.28	0.71	$\equiv 1.0$	12
0.17 ± 0.02 -0.03	0.22 ± 0.07 -0.02	0.71 ± 0.01	$\equiv 1.0$	9 and R.O. Pepin, private communication

presence of magnesium isotope anomalies, again in carbonaceous meteorites, which, too, have been interpreted as being 'non-solar'. Theoretical models of primitive solar nebulae¹⁹ and the collapse of rotating protostars (D.C.B. and P. Bodenheimer, to be published) indicate that at heliocentric distances $\gtrsim 1\text{--}5$ AU, nebular temperatures would not have been sufficient to degas quantitatively interstellar dust grains present in the primitive nebula. Thus, the available evidence indicates that a fraction of the solid matter in the early solar system retained the elemental and isotopic compositions characteristic of their place of formation.

Generally, r-process nucleosynthesis is imagined to imply the rapid addition of neutrons to nuclei to synthesise elements beyond the iron peak. Typically, one is concerned with neutron densities $n_n \sim 10^{24} \text{ cm}^{-3}$ and temperatures $\sim 10^9$ K. Under such conditions, the time scale for the addition of neutrons, $\tau_n = (n_n v_T \sigma_n)^{-1}$ where v_T is the mean thermal speed of the neutrons at temperature T and σ_n is the capture cross section of a given nucleus for neutrons, is much less than the time scale (τ_β) for β^- decay of the nucleus. In this situation, equilibrium is established between (n, γ) and (γ, n) reactions, with β^- decay providing a small perturbation or 'leakage' to the equilibrium established by the neutrons and photons. Such a picture leads to an abundance curve with a peak at mass 130 (ref. 20), shown schematically in Fig. 1. The peak is associated with a magic number ($N = 82$) of neutrons.

Fig. 2 Relative abundance ($^{136}\text{Xe} = 1$) of the xenon isotopes ^{134}Xe , ^{132}Xe and ^{131}Xe . *a*, *b*, Curves for r-process and s-process, respectively, obtained by renormalising those in Fig. 1 so that their abundance peaks occur at mass 136, rather than mass 130 and 138 respectively. *c*, Composition of R xenon.



In the other limit, $\tau_\beta \ll \tau_n$, nucleosynthesis (s-process) proceeds along the valley of β -stability. Again, one finds that the effect of the closure of shells containing magic numbers of neutron appears in the form of a peak in the abundance curve at 82 neutrons and a mass of 138 a.m.u. (ref. 20) (Fig. 1). Note that both abundance peaks are steep and smooth because of the smooth variations in nuclear properties near the magic number of neutrons.

Figure 2 shows the abundance curves one would expect if the r-process and s-process peaks occurred at $A = 136$ rather than $A = 130$ and 138 respectively. The curves are normalised so that $^{136}\text{Xe} = 1$. Also shown is the abundance curve defined by R xenon. The R xenon curve falls between r-process and s-process abundance curves at all isotopes. If the r-process was altered to give rise to a peak at $A = 136$, it would be steeper than the plotted r-process abundance curve. In the limit, it must converge to the s-process abundance curve. Thus on a qualitative basis, the isotopic structure of R xenon is consistent with an r-process nucleosynthesis which produced an abundance peak at $A = 136$.

The physical condition required to shift the r-process abundance peak to larger values of A is basically that τ_n be increased. This allows the β^- decay to proceed further up the magic neutron ladder, at $N = 82$, than is the case for the 'standard' r-process nucleosynthetic chain. In fact, ^{136}Xe lies on the β^- decay ladder at $N = 82$ and is the first stable nucleus encountered by the ladder. The increase in τ_n can be brought about by lowering n_n and/or T . Preliminary estimates of the range in n_n and T required suggest a low density, high temperature regime such as one might encounter in a shock wave traversing the atmosphere of a supernova precursor.

It has been suggested²¹ that many of the p-process (proton-rich) nuclei are created in a supernova shock. If so, an intimate mixing of p-process nuclei and R xenon might result. The newly discovered evidence for such a mixing in carbonaceous meteorites would be a natural consequence of the modified r-process hypothesis.

One might expect manifestations of the modified r process in other elements. In particular, effects should be seen at three of the isotopes of krypton, namely ^{86}Kr , ^{84}Kr , and ^{83}Kr . As with ^{136}Xe , ^{86}Kr is the first stable nucleus encountered by a β^- decay ladder at a magic neutron number ($N = 50$). The magnitude of the attendant krypton effect cannot be estimated without more detailed calculations.

The abundance ($\text{cm}^3 \text{ STP g}^{-1}$) of R xenon does not lead to any particular problem (as is the case for the fission hypothesis). Assuming that the concentration of ^{136}Xe (R xenon) in dust grains is similar to typical xenon concentrations in meteorites ($\sim 10^{-8} \text{ cm}^3 \text{ STP g}^{-1}$), only $\sim 1\%$ of the material in carbonaceous meteorites need contain R xenon to explain the observed bulk abundance data. The correlation of ^{136}Xe (R xenon) with trapped ^{132}Xe would be expected if dust grains which contain R xenon mix with varying amounts of dust grains formed in the solar nebula. A similar correlation exists between trapped ^{36}Ar and the singular neon component discussed previously¹⁵, and has been interpreted in the same manner as the ^{132}Xe :R xenon correlation^{15,22}.

Perhaps the most obvious future theoretical effort would be to vary the r-process nucleosynthesis parameters to give rise to an abundance peak at ^{136}Xe , and compare the resulting spectrum with that of R xenon. It is likely that requiring the abundance peak to occur at ^{136}Xe is a necessary, but not sufficient constraint on the nucleosynthetic calculations. One would like to determine the sensitivity of the calculated spectrum to variations in the controlling physical parameters (T , n and so on). If, within the uncertainty in the calculated spectrum, agreement is obtained between the experimental and theoretical spectra, one would like to know the astrophysical environment, or range of environments, likely to engender R xenon. In this connection, will the same environment(s) give rise to P xenon?

The experimental data available to date have primarily come

from stepwise heating experiments on bulk samples. This type of experiment provides a quantum jump over simple bulk analysis in the detailed knowledge one obtains from a sample. The state of the art now, however, requires a further quantum jump in information content per experiment, namely stepwise heating experiments on various discrete mineral phases or assemblages from a meteorite. It would seem particularly important to do rare gas analyses of at least neon and xenon in a given sample, and to have oxygen and magnesium isotope analyses done on aliquots of the rare gas samples. The questions are: do R xenon, neon E (ref. 15) and the oxygen and magnesium isotope anomalies occur together, with what frequency and in what minerals? Are P xenon and R xenon well mixed, and if so is it on a microscopic scale (individual mineral phases and/or grains) or a macroscopic (in bulk) scale?

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- ¹ Phinney, D. L. *Meteoritics*, **4** (1973).
- ² Manuel, O. K., Henneke, E. W., and Sabu, D. D., *Nature*, **240**, 99 (1972).
- ³ Alexander, E. C. Jr, Lewis, R. S., Reynolds, J. H., and Michel, M. C., *Science*, **172**, 837 (1971).
- ⁴ Reynolds, J. H., and Turner, G., *J. geophys. Res.*, **69**, 3263 (1964).
- ⁵ Hoffman, D. C., and Hoffman, M. M., *A. Rev. nucl. Sci.*, **24** (in the press).
- ⁶ Anders, E., and Heymann, D., *Science*, **164**, 821 (1969).
- ⁷ Rao, M. N., *Nucl. Phys. A*, **140**, 69 (1970).
- ⁸ Takaota, N., *Mass Spectroscopy*, **20**, 287 (1972).
- ⁹ Phinney, D. L., thesis, Univ. Minnesota (1971).
- ¹⁰ Pepin, R. O., in *Origin and Distribution of the Elements*, 379 (Pergamon, Oxford, 1968).
- ¹¹ Eugster, O., Eberhardt, P., and Geiss, J., *Earth planet Sci. Lett.*, **1**, 99 (1966).
- ¹² Marti, K., *Meteoritics*, **5**, 208 (1970).
- ¹³ Sabu, D. D., Henneke, E. W., and Manuel, O. K., *Nature*, **251**, 21 (1974).
- ¹⁴ Black, D. C., and Pepin, R. O., *Earth planet. Sci. Lett.*, **6**, 395 (1969).
- ¹⁵ Black, D. C., *Geochim. cosmochim. Acta*, **36**, 377 (1972).
- ¹⁶ Clayton, R. N., Grossman, L., and Mayeda, T. K., *Science*, **182**, 485 (1973).
- ¹⁷ Gray, C. M., and Compston, W., *Nature*, **251**, 495 (1974).
- ¹⁸ Lee, T., and Papanastassiou, D. A., *Geophys. Res. Lett.*, **1**, 227 (1974).
- ¹⁹ Cameron, A. G. W., and Pine, M. R., *Icarus*, **18**, 377 (1973).
- ²⁰ Cameron, A. G. W., *Space Sci. Rev.*, **15**, 121 (1973).
- ²¹ Hoyle, F., and Fowler, W. A., *Nature*, **241**, 384 (1973).
- ²² Black, D. C., in *On the Origin of the Solar System*, 236 (CNRS, Paris, 1972).

The beginning of a new cycle of solar activity

ON November 15, a high latitude sunspot marked the beginning of a new cycle of solar activity. The present cycle is on the decline and is approaching its minimum, a new cycle thus starting before the old one has completely disappeared. At this time the sunspots belonging to the old cycle originate near the equator, whereas those of the new one occur at higher latitudes.

The latitude of the sunspot observed was $+37^\circ$, and it was 60° from the central meridian. It could be seen clearly the following day but not on November 17, when it crossed the western limb. The first spots of a new cycle usually appear more than a year before the coming minimum, so because the present activity is still relatively high, the minimum cannot be expected to occur before 1976.

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Mathematical prediction of climatic change

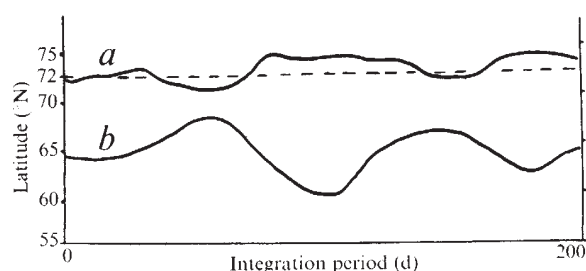
A WIDESPREAD and increasing degree of attention is being paid to the problem of understanding the physical factors which lead to climatic change. Analysis of past weather data¹ derived from a wide variety of sources has shown how the main characteristics of climate have changed over recent centuries. If, however, scientific theories are to be developed, it is desirable that mathematical models of climate be constructed. These must include at least the primary global scale dynamics of the atmosphere and should allow the operation of necessary long time-period integrations on available computers.

The primary physical factor responsible for initiating the stirring of the atmosphere into its ever changing assembly of the cyclonic waves and anticyclonic systems is the meridional distribution of solar radiation, $S(\theta)$, where θ is the latitude. The response of the atmosphere to a given $S(\theta)$ and to the associated requirement of balancing the heat budget is seen in the continuously created train of weather systems which strive to rectify the imbalance; these systems are important agencies in determining climate. The sensitivity of climate to changes in solar radiation or changes in atmospheric transparencies should, therefore, be investigated.

In approximate calculations Budyko² used a system of empirical equations describing an assumed static balance (in a temporal sense) between the residual vertically integrated and zonally averaged annual radiation heating at a given latitude and the vertically integrated meridional flux divergence of heat. He assumed that his empirical heating functions were still valid when the total solar radiation was altered by a series of small percentages. An iterative computer procedure allowed Budyko to calculate the associated shift in the meridional distribution of mean atmospheric temperature, atmospheric and surface albedo, and the new mean, zonally averaged, simulated ice edge position. The primary atmospheric physics on a global scale is contained in Budyko's model (although rather crudely) but he assumed a static atmospheric balance, purporting to represent an asymptotic, annual average situation, corresponding to a prescribed solar heating; and there is no indication of how sensitively the calculated results depend on the mathematical forms of the empirical functions used. Consequently, his main result, suggesting that a 1.5% reduction in incoming solar radiation (or an equivalent change in atmospheric transparency) would lead to a new ice age, has met with scepticism. An extended form³ of the calculation has indicated that as much as a 5% reduction in solar radiation would be needed before large scale movement of the ice edge would occur.

These simplified static calculations, however, tell us nothing about time variations around the mean values of the large scale structure of atmospheric dynamics; indeed it may be completely incorrect to assume a stable balance of the averaged flow system for a perturbation of solar heating greater than some

Fig. 1 Position (latitude) of the zonally averaged simulated ice edge as a function of time over a 200-d period. a, Solar heating values representing the present decade; b, a 3% reduction in solar heating.



critical value. For example, with small reductions in solar heating the zonally averaged ice-snow edge in the model may oscillate about its mean latitudinal position with a small spatial amplitude, as in a dynamically stable state; but with larger reductions the amplitude may become larger and there may occur a progressive movement towards the equator of the zonally averaged snow-ice position.

We have developed a time-dependent mathematical numerical model containing only the global scale dynamics, which simulates observed oscillations of the total atmospheric eddy kinetic energy associated with variations in the intensity of cyclonic systems. Basically, it is a modification of that introduced by Phillips⁴, and used⁵ in studies of the dynamics of anticyclonic blocking. The effects of standing waves on the planetary scale created by mountain barriers are not included and the effects of heating and friction in the model are taken as zonally averaged over continents and seas. The heating function, however, is not fixed geographically, as in the previous model, but is dependent on model atmospheric temperature, and a surface thermal balance equation is used, which predicts model surface temperatures. The very long time period integrations essential to theoretical studies of climate are practical from a computing point of view: only 10 min computing time is required to carry out approximately 150-d integrations on the Harwell IBM 360/195 computer. The results obtained predict a scientifically interesting characteristic of the dynamics of climatic change and also show that numerical experiment can be applied profitably to this important practical question.

The model uses an initial meridional distribution of solar heating, $S(\theta)$, equivalent to annually averaged and zonally averaged observed heating values. After a long integration period of about 300 d, the model apparently reaches an asymptotic 'equilibrium' dynamic state with the simulated snow-ice edge oscillating about a mean latitude of roughly 72°N. This simulation is based on the predicted model surface temperature, and includes an associated albedo distribution taken from observational data; an empirical time lag could have been built into the representation but temporal variations in the position of the edge are slow compared with the time step of integration. As such a representation would have involved further extensive computing it has therefore been omitted at this stage. The moving position of the edge is shown in Fig. 1 for a 200-d period. At the end of that period S was reduced by 0.5% and the computation continued for a further 400-d period. The calculated mean position of the ice edge over that period coincided with the result obtained previously using our statical method³. The process was repeated for successive small reductions, δS , in S . When δS approached 3% the westerly, mid-latitude, cyclonic wave type of flow became punctuated increasingly by low latitude, Hadley-cell circulations which penetrated to higher latitudes for increasing periods of time. Indications of this characteristic of climatic change have already been noted by Lamb¹. At the same time, the spatial amplitude of the oscillating edge increases sharply as δS approaches 3% (Fig. 1) although the mean position is at about the latitude predicted by statical calculations³. In the type of model used by Everson and Davies, latitude does not correspond exactly to latitude on the spherical Earth although there is a close approximation.

We deduce from these results that, although the computed movement of the zonally averaged ice edge follows roughly the results of previous statical calculations³, the dynamic model shows signs of rapidly increasing instability (reflected in the increasing amplitude of oscillation of the simulated ice edge) at each mean equilibrium position, as solar heating is reduced by up to 3%. The model is known not to reproduce the low latitude (Hadley cell) mean meridional circulations very well and several important feedback processes are omitted; there is a pressing need to study the dynamics of climatic change with more sophisticated model systems including a realistic oceanic-atmospheric link and representations of mean seasonal movements. Our initial results form, however, a step forward

from previous calculations based on statical models, and show that, although equilibrium solutions (of the Budyko type) exist with as much as 5% reduction in solar heating, their stability becomes very suspect as 3% reduction values are approached, and the atmospheric circulation changes fundamentally. This suggests that there is a danger that a further increase will trigger off a progressive movement of the snow-ice edge towards the equator. Calculations beyond this stage have not been carried out, as we feel that the premises of the model are unlikely to apply.

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¹ Lamb, H. H., *Climate: Present, Past and Future*, 1 (Methuen, London, 1972).

² Budyko, M. J., *Tellus*, 5, 611 (1969).

³ Gordon, H. B., and Davies, D. R., *Q. Jl R. met. Soc.*, 100, 123 (1974).

⁴ Phillips, N. A., *ibid.*, 82, 123 (1956).

⁵ Everson, P. J., and Davies, D. R., *ibid.*, 96, 404 (1970).

Detection of elastic strainfields caused by fault creep events in Iran

SLIP movements along active faults may either occur violently, and generate earthquakes, or gradually in a little understood process called fault creep¹⁻⁵. An interesting feature of fault creep is that it is frequently episodic rather than continuous and the episodes of movement propagate along the fault¹. The mathematics, but not the physics, of the process are identical to those of crystal dislocations⁶.

Although displacements across faults have been monitored carefully for some time^{4,5}, there has been almost no study of the transient strains that must be associated with the passage of each creep event. Frank⁷ has discussed the strain-time histories that can be expected theoretically during typical strike-slip creep events, and we here follow his formulation.

For a single, infinitely long, edge dislocation of slip strength, b , normal to the surface in a homogeneous semi-infinite half-space⁷:

$$e_{\phi} = \frac{-b}{4\pi y} \left\{ (1-2\nu) \frac{1}{1+X^2} + (1+2\nu) \times \right. \\ \left. \times \left[\cos 2\phi \frac{2X^2}{(1+X^2)^2} + \sin 2\phi \frac{X(1-X^2)}{(1+X^2)^2} \right] \right\} \quad (1)$$

where ν is Poisson's ratio, e_{ϕ} is the linear strain at a point x, y , $X = x/y$, and the surface trace of the fault lies along the x -axis with y chosen such that in a right-handed coordinate system z is positive upward. ϕ is the angle between strainmeter and the x -axis, and increases in an anticlockwise fashion looking down.

A screw dislocation parallel to the x axis at a depth d gives a strain of:

$$e_{\phi} = - \frac{b}{4\pi y} \frac{4D}{1+D^2} \sin\phi \cos\phi \quad D > 0 \quad (2) \\ = 0 \quad D \leq 0$$

Where $D = d/y$. The function $-b/4\pi y$ indicates that the strain magnitude is proportional to the size of the dislocation and decays inversely with distance from the fault.

Equations (1) and (2) describe the spatial variation of strain resulting from static dislocation. If, however, the dislocation moves with a velocity which is slow compared with the shear

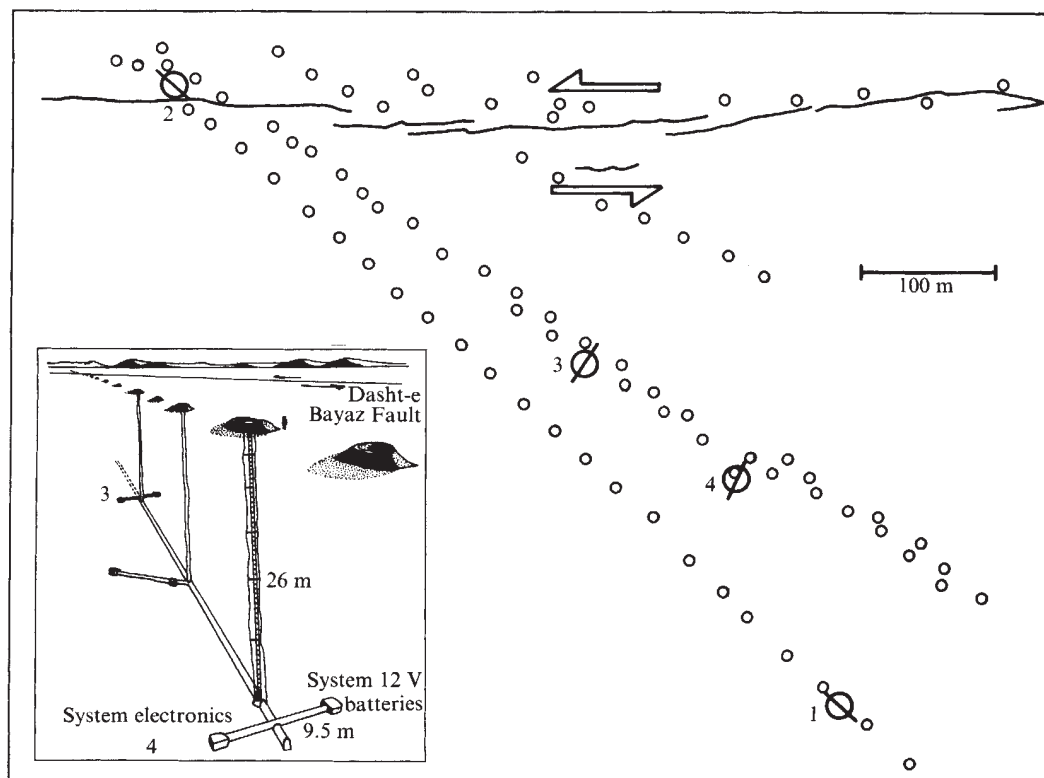


Fig. 1 The location of the strainmeters superimposed on a map of the Dasht-e-Bayaz fault break⁸. Open circles, vertical well shafts connecting qanats to the surface; 1-4, positions of strainmeters—the symbols indicate orientation. The insert shows a diagram of the qanat system containing instruments 3 and 4.

wave velocity, the strain as a function of time may be obtained by substituting

$$X = (x_0 - ut)/y$$

in equation (1) and

$$D = (d_0 - wt)/y$$

in equation (2). The parameters x_0 and d_0 refer to the position of the dislocation at time, $t = 0$, and u and w are the x and z components of the velocity. As equation (1) and (2) are not invariant with respect to a reversal in the velocity, the strain as a function of time may be used to determine the direction of propagation of a dislocation.

The instruments on which we have observed creep related strains are situated in eastern Iran near to the fault break⁸ of the 1968 Dasht-e-Bayaz earthquake. They are sited in horizontal underground irrigation tunnels called qanats (Fig. 1). These connect to the surface at regular intervals through vertical well shafts. Instruments 3 and 4 are installed in a completely separate qanat system (insert, Fig. 1) from the array comprising instruments 1 and 2. Within an array the instruments share the power supply and the recording system, but there is no electrical connection between separate arrays. These circumstances greatly reduce the possibility that a freak instrumental effect will be observed.

Column *a* of Fig. 2 shows data from the four instruments. Instruments 1 and 2 were recorded on one chart paper and 3 and 4 on a second. To record the data (Fig. 2*a*) the instruments use pressure-sensitive paper marked by a galvanometer needle that is struck every 10 s by a chopper bar. The sampling interval for instruments 1 and 2 is much longer than that for instruments 3 and 4 which gives rise to discontinuous traces (Fig. 2*a*). Tides are visible on the records and long spans of tidal data from instruments 1 and 2 and a third now defunct instrument in the same qanat have been analysed. The results (Fig. 2*a* and *b*), indicate that no significant rigidity anomalies are apparent in the region of the arrays. The very large strain magnitude observed on instrument 2 (Fig. 2*b*) excludes the possibility that a pressure effect has

been observed⁹. The temperature coefficients of the instruments are less than 10^{-7} per $^{\circ}\text{C}$ and the temperature in the instrument 2 site remains constant to within 0.1°C over periods of days. The excursion is therefore too large and too rapid to be a thermal effect.

Predicted strain signals for an edge dislocation—equation (1)—propagating eastwards ($u = 200 \text{ mm s}^{-1}$; Fig. 2*c*), and westwards ($u = -200 \text{ mm s}^{-1}$; Fig. 2*d*) along the fault, show clearly that an eastward, but not a westward, propagating creep event fits the data rather well. That direction of propagation is also suggested by the fact that the event first appears on instrument 2. Furthermore, the velocity of 200 mm s^{-1} both produces strain records like those observed, and accounts for the different onset times on the different instruments.

The strain records for instruments 3 and 4 for 12 h before the observation of the events described here, are significantly disturbed from the normal tidal signal for the same instruments (Fig. 3*a*). For comparison, a short section of typical tidal data for instrument 3 is shown (Fig. 3*b*). Consideration of the predicted strain record (Fig. 3*c*) for these instruments for a downward propagating (equation (2): $w = -200 \text{ mm s}^{-1}$) screw dislocation suggests that some of the signal observed by instruments 3 and 4 in this 12-h period might have been produced by downward propagating creep events. These events are not, however, clearly visible on the records of instruments 1 and 2 possibly because of the high noise level or the long sampling interval.

The present network of four instruments is insufficient to permit determination of the depth and extent of the creeping surface. The event interpreted as having been caused by an edge dislocation was, however, detected to the west of instrument 2 and was still visible until it was to the east of instrument 1. It must, therefore, have propagated more than 800 m. It is of particular interest that this same event can be explained by an infinite dislocation with a slip strength, b , of less than $10 \mu\text{m}$. If the depth of the edge dislocation is limited by allowing it to generate or consume a trailing or leading screw dislocation, the size of the required slip and the form of the predicted signals are not altered significantly, unless the whole process is restricted to a few tens of metres near the surface. That is

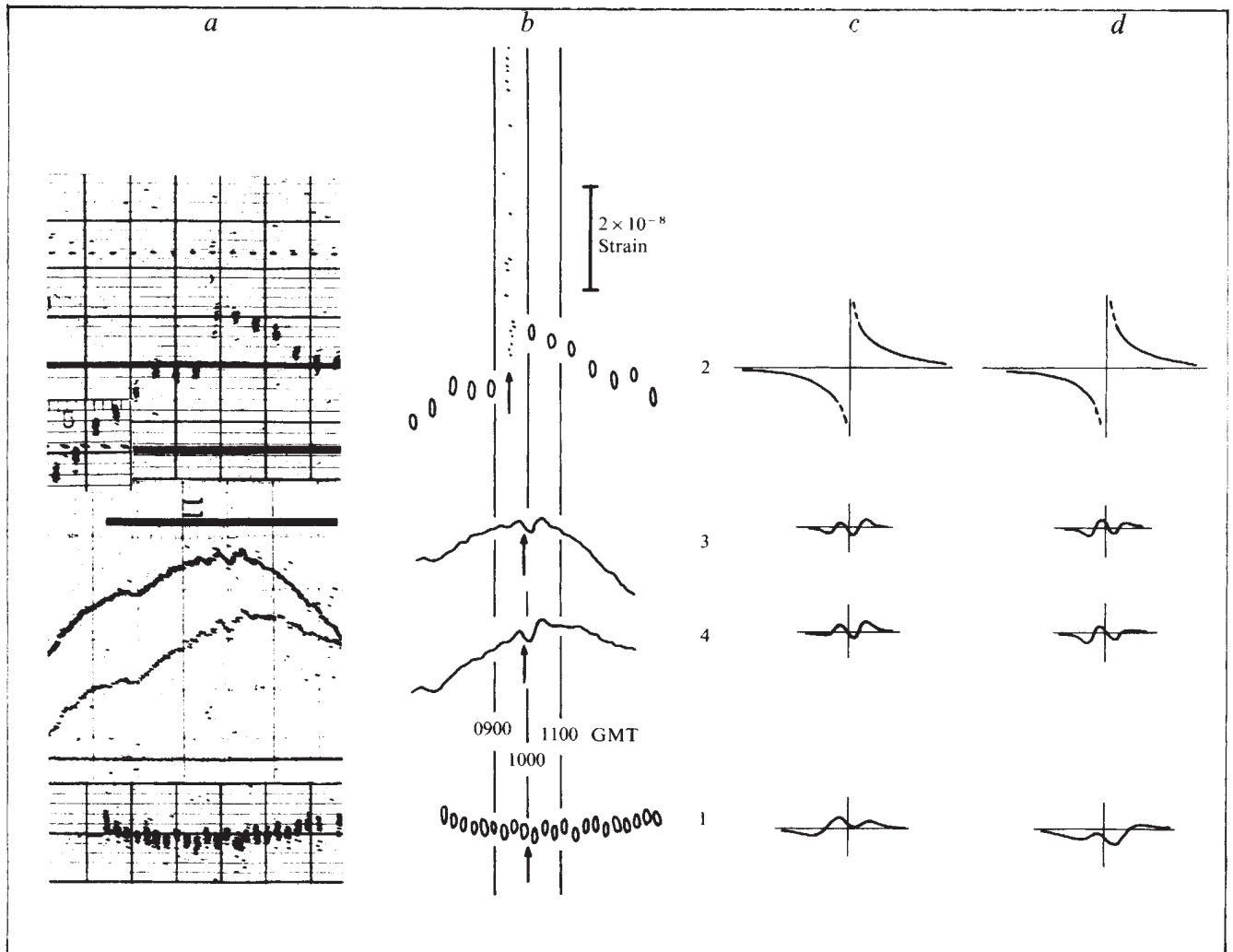


Fig. 2 *a*, Data recorded by the strainmeters between 0900 and 1000 on April 24, 1974 (the numbers in the centre of the figure correspond to the numbers of the instruments). Traces 1 and 2 were written by an earlier and noisier design of strainmeter than were traces 3 and 4. *b*, tracings of the data shown in column *a*. The large excursion of the trace of instrument 2 (which is closest to the fault) is easily visible on the original record, though not on the reproduction in column *a*. *c, d*, Predicted strain records (*c*, for an eastward propagating edge dislocation; *d*, for a westward propagating edge dislocation). The vertical lines in columns *c* and *d* correspond to $X = 0$ in equation (1), and the arrows on the data tracings (column *b*) correspond to where the vertical lines overlay when *c* is fitted to *d*. The horizontal scales are everywhere the same. The velocity of the creep event in *b* and *c* is 200 mm s^{-1} . The vertical scales are the same and correspond to the scale bar. The amplitudes of the predicted signals have been chosen to facilitate comparison with the data. They are within $\pm 20\%$ of the amplitudes predicted by a dislocation of slip strength, $b_s = 10 \mu\text{m}$ except in the case of instrument 1 which, for clarity, has been exaggerated by a factor of 2.

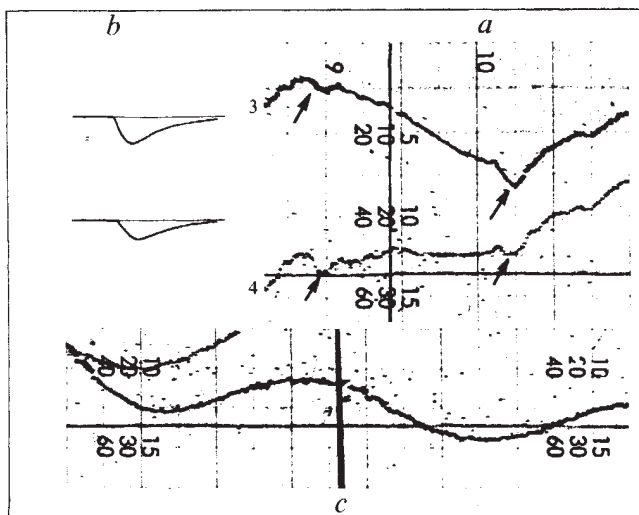


Fig. 3 *a*, Records from instruments 3 and 4 for 12 h before the data displayed in Fig. 2; *b*, predicted form for downward propagating ($w = -200 \text{ mm h}^{-1}$) screw dislocations; *c*, predicted form for a typical span of tidal data recorded at the same site on instrument 3.

clearly unlikely for an event which propagated at least 800 m. Models which invoke exotic departures of rigidity from an homogeneous half space are excluded by the consistency of the tidal data.

Although a creep event was previously observed on the same fault by a creepmeter 5 km from the strain array, and even though geodetically measured movement is believed to be occurring (J. Brander, personal communication), no creep events were detected in association with the strain signals. That is hardly surprising in view of the fact that 10 μ m is well below the threshold level of our own and other people's creepmeters. (Californian creep events are often 10^3 times larger.)

A further point of interest is that our predicted signals do not resemble those of typical creep events as written by creepmeters. In that respect they differ from strain records obtained at Stone Canyon¹⁰. Though it is possible that those records were produced by very localised fault creep¹⁰, or by local slumping, all of our strainmeter designs have produced records that resemble creep events on creepmeters. Our instruments have been installed at stable sites in England and we believe that these 'events' are caused by instrumental or siting problems because they usually occur randomly on closely grouped instruments.

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- ¹ King, C. Y., Nason, R. D., and Tocher, D., *Phil. Trans. R. Soc.*, **A274**, 355 (1973).
- ² Burford, R. O., Allen, S. S., Lamson, R. J., and Goodreau, D. D., in *Tectonic Problems of the San Andreas Fault System*, *Publ. geol. Sci.*, **13**, 268-274 (Stanford University, California, 1973).
- ³ Nason, R. D., and Weertman, J., *J. geophys. Res.*, **78** (32), 7745 (1973).
- ⁴ Nason, R. D., Philippson, F. R., and Yamashita, R. A., *Catalogue of Creepmeter Measurements in Central California from 1968 to 1972* (United States Geological Survey, National Centre for Earthquake Research, Menlo Park, California, 1974).
- ⁵ Yamashita, R. A., and Burford, R. O., *Catalogue of Preliminary Results from an 18-station creepmeter network along the San Andreas fault system in Central California for the time interval June 1969-June 1973* (United States Geological Survey, National Centre for Earthquake Research, Menlo Park, California, 1974).
- ⁶ Nabarro, F. R. N., *Theory of Crystal Dislocations* (Oxford University Press, London, 1967).
- ⁷ Frank, F. C., *Phil. Trans. R. Soc.*, **A274**, 351 (1973).
- ⁸ Tchalenko, J. S., and Ambraseys, N. N., *Bull. geol. Soc. Am.*, **81**, 41 (1970).
- ⁹ Bilham, R. G., King, G. C. P., and McKenzie, D. P., *Geophys. J. R. astr. Soc.*, **37**, 237 (1974).
- ¹⁰ Stewart, R. M., Bufo, C. G., and Pfluke, J. H., in *Tectonics Problems of the San Andreas fault system*, *Publ. geol. Sci.*, **13**, 286-293 (Stanford University California, 1973).

Lineations in the Magnetic Quiet Zone of the North-west Atlantic

A MAGNETIC and seismic reflection survey in the Magnetic Quiet Zone shows that basement topography and magnetic anomalies are lineated parallel to the boundary of the quiet zone. Two narrow zones of reversed polarity are identified. The results imply that the zone boundary is an isochron separating a period of seafloor spreading in which rapid geomagnetic reversals occurred from a period in which few reversals occurred.

There have been many attempts to explain the presence of the Magnetic Quiet Zone which lies seaward of the continental shelves bordering the North Atlantic. In terms of crustal layer thickness and basement topography the quiet zone resembles normal oceanic crust. It is, however, unusual because the magnetic anomalies have a low ampli-

tude within the area. The following explanations for the attenuated nature of the anomalies have been suggested: (1) magnetisation during a time interval characterised by the absence of geomagnetic polarity reversals¹⁻³; (2) original magnetisation with very low inclinations acquired in equatorial magnetic latitudes during the Triassic⁴; (3) hydrothermal alteration resulting in reduced magnetisation⁵; and (4) intrusives rather than extrusives as the dominant component of layer 2 (ref. 6). These have been reviewed by Pitman and Talwani⁷.

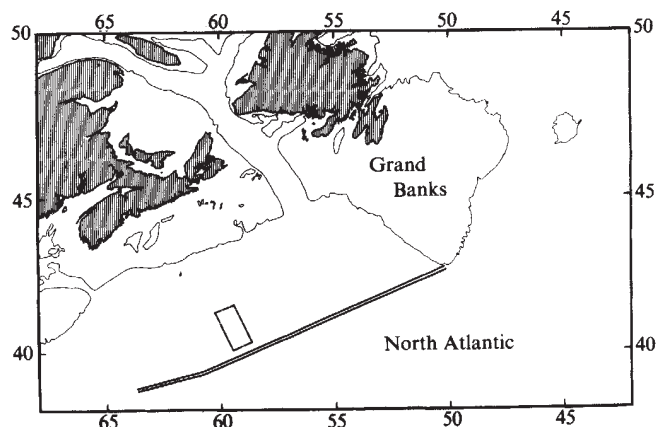


Fig. 1 Location chart showing the area of the survey (dashed lines) and the quiet zone boundary (double solid lines).

In 1972, detailed magnetic, seismic reflection and sonobuoy seismic refraction studies were carried out within an area 150 km $\times$ 70 km in the quiet zone of the North-west Atlantic (Fig. 1). The purpose of the experiment was to test the hypotheses concerning the nature and origin of this area. The measurements were made from CSS Hudson at a track spacing of less than 7 km (Fig. 2). Using LORAN-C in the range-range mode together with satellite navigation, the survey lines could be positioned to an accuracy of 200 m. The observed magnetic anomaly field was compared with a field calculation based on a layer of constant thickness with the observed topography of oceanic basement, so that the presence of magnetic reversals, if any, could be detected. The results provide estimates of the magnetisation of layer 2 in the quiet zone and indicate the direction of lineation of the magnetic anomalies and of topography of the top of layer 2.

Many of the experimental methods used in this study have been described previously⁸. The magnetic data were corrected for diurnal variations which are of the same amplitude, approximately 60 gamma, as the spatial variations of the magnetic anomaly field. A magnetometer manufactured by Sander Geophysics was moored in the survey area and the data from this and observations at the magnetometer station at Bedford Institute, Dartmouth, Nova Scotia, were used to make the diurnal corrections.

During the survey no unusual magnetic disturbances occurred and the r.m.s. crossover error of the corrected total magnetic field is 4.5 gamma. The anomaly field was obtained by subtracting the 1965.0 IGRF regional magnetic field⁹ plus a constant shift of 173 gamma. The latter is necessary if a mean value of zero for the anomaly field is desired. The magnetic data were then contoured and the resulting contour chart is shown in Fig. 2.

The seismic reflection data were collected along fewer tracks than the magnetic measurements (Fig. 2). Oceanic

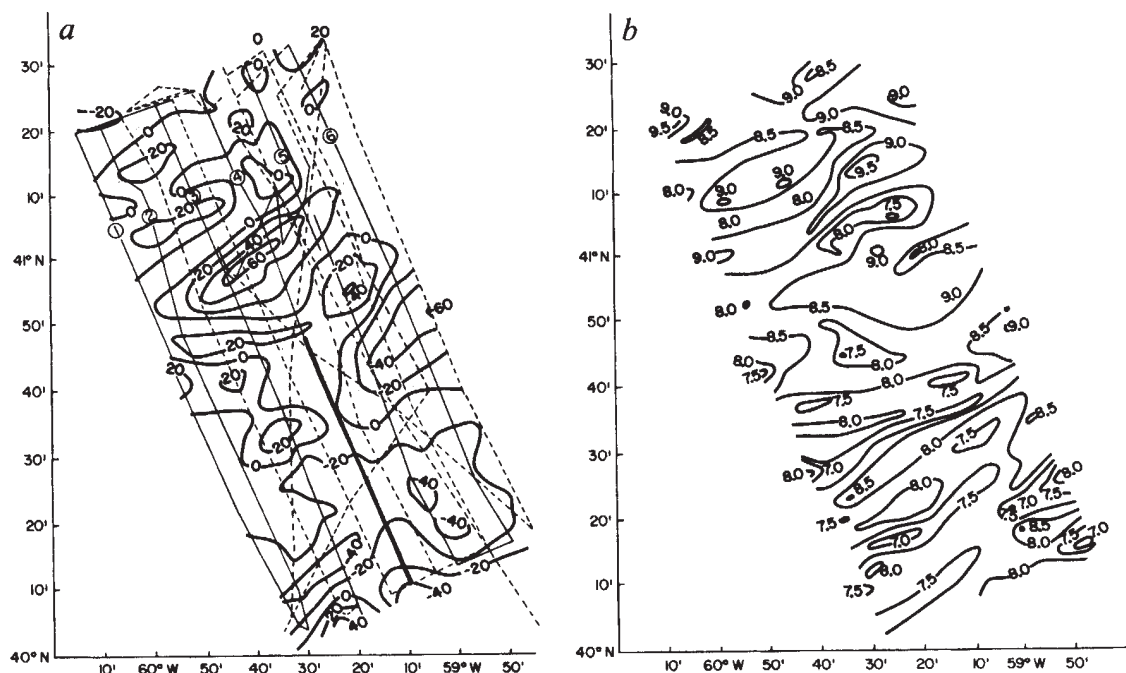
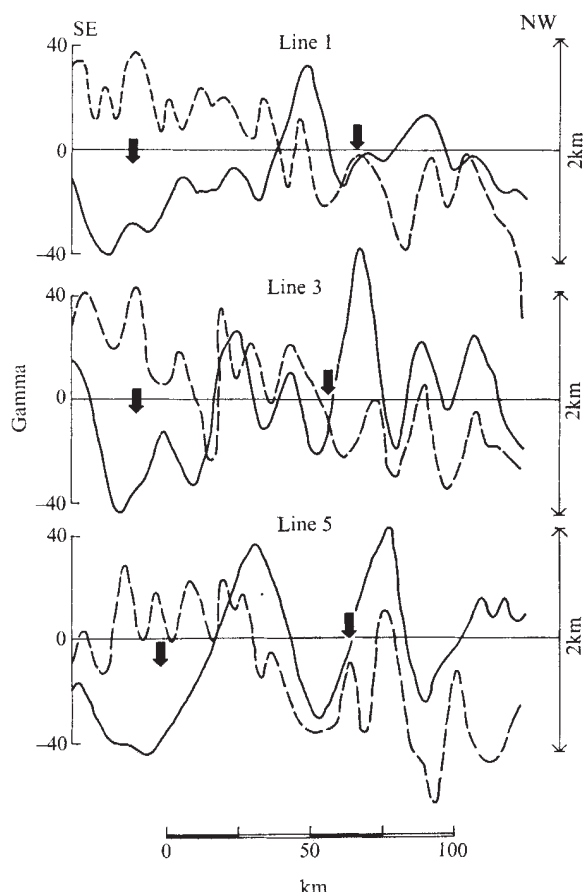


Fig. 2 Contour charts: *a*, the magnetic anomalies; *b*, the basement topography, showing the tracks along which data were collected. ---, Tracks along which only magnetic data were collected; —, tracks along which magnetic and seismic reflection and refraction data were obtained. The circled numbers on the solid lines are line identification numbers, used in Fig. 3. The magnetic contour values are given in gamma and the topography values are in km below sea level.

Fig. 3 The magnetic anomalies (—) and corresponding basement topography (---) along three lines. The line numbers are those indicated in Figure 2. The arrows denote positions at which there is a negative correlation between basement topography and the magnetic anomalies.



basement was invariably observed although in some instances it was difficult to determine accurately the top of this layer because of the rough nature of the reflector which caused side echoes. The depth to basement in one-way travel time was converted into distance using a mean velocity of 2.0 km s^{-1} for the sedimentary column down to horizon β and 3.0 km s^{-1} for the remainder of the section. This velocity is based on the sonobuoy results.

The contour maps of the magnetic anomalies and of the basement topography show that both are lineated approximately parallel to the quiet zone boundary (Figs 1 and 2). The magnitude of the total magnetic anomaly field varies from about 60 to -40 gamma, and wavelengths of the prominent anomalies are about 30 km, which is similar to the wavelengths of anomalies within the disturbed zone. With respect to its lineated appearance and its relief of 1 km or more, the character of oceanic basement is similar to the character of the exposed basement rocks near the crest of the mid-Atlantic ridge. This suggests a seafloor spreading origin for the quiet zone. The magnetic lineations could be a reflection of the basement trends and could also be influenced by geomagnetic reversals.

In interpreting the results, answers to three main questions concerning the quiet zone were sought: First is there evidence that geomagnetic reversals have occurred and, if so, how frequent are they? Second, what is the magnetisation of oceanic basement? Third, was the oceanic crust formed at low geomagnetic latitudes?

To detect the presence of reversals, correlations of the magnetic anomalies with topography were made. Figure 3 shows the basement topography and magnetic anomalies along three tracks which cross the survey area. The topographic profiles show more variation than the magnetic anomalies. The attenuation of the shorter wavelength components of the magnetic anomalies is caused by the large separation (about 8 km) between the magnetic source and the level of measurements. Figure 3 indicates that the topography and the magnetic anomalies correlate well in terms of the positions of the peaks and troughs and suggests that

most of the magnetic anomalies may be caused by basement topography. But at two locations on each of the profiles there is a negative correlation. At the positions shown by the arrows in Fig. 3 the basement relief is insufficient to cause the magnetic lows. This is consistently observed on all of the NW-SE tracks. So there are two strips trending parallel to the magnetic lineations where magnetic data and basement topography suggest that the basement rocks must possess reversed polarity. The good correlation between topography and magnetic anomalies elsewhere shows two significant characteristics: First, the region is predominantly underlain by oceanic basement with remanent magnetisation in the normal direction, and second the larger part of the anomalies must be caused by variations in basement topography.

A theoretical model of oceanic basement describing the topographical variations within the survey area was constructed using rectangular blocks, $1.8 \text{ km} \times 5.4 \text{ km} \times 1.8 \text{ km}$ thick. This thickness has been used by others⁴, so comparisons of results can be made easily. The blocks were aligned so that the 5.4 km length lay in the direction of the lineations and the 1.8 km length perpendicular to it. The heights of the blocks were raised or lowered to simulate the observed topography. Calculations of the magnetic field arising from this three-dimensional model were made for various values of magnetisation, magnetic inclination and strike, and for various widths of the zones of reversed polarity. With these results, an estimate of the magnetisation of the basalts can be obtained.

We computed the three dimensional magnetic anomalies using declinations and inclinations consistent with both Jurassic and Triassic palaeomagnetic pole positions for North America. A remanent magnetisation value of 1.1×10^{-2} e.m.u. cm^{-3} gave the best fit in the zones of normal polarity and for the sake of simplicity in the model, the same value was used in the two zones of reversed polarity shown in Fig. 3. In the latter case, the high magnetisation value imposed a limit on the width of the reversed zones in the model of 1 km. A more detailed model with lower values of reversed magnetisation would undoubtedly allow the reversed zones to be wider, particularly in the southernmost zone. Simplified contour maps of the computed magnetic

anomalies for the Triassic and Jurassic models and for the observed data are presented in Fig. 4. These were obtained by shading regions above 20 gamma and below -20 gamma. Although the correspondence between the observed and computed maps is not exact, the main belts of positive and negative anomalies appear in each. The Jurassic model gives the best fit to the observed anomalies in the northern part of the area where reversals do not affect the magnetic anomaly pattern. This is additional evidence to support the argument that low magnetic latitudes during the Triassic are not required to explain the magnetic anomalies in the quiet zone and further suggests that the crust is Jurassic in age. Although the models could be varied to resemble the observations more closely, the numerous geological variations which have not been included in the model together with the errors in the measurements make these refinements unrealistic.

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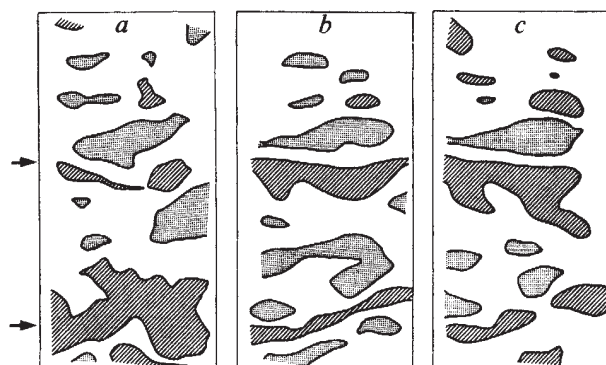
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- ¹ Emery, K. O., Uchupi, E., Phillips, J. D., Bowen, C. D., Brunce, E. T., and Knott, S. T., *Bull. Am. Ass. petrol. Geol.*, **54**, 44-108 (1970).
- ² Heirtzler, J. R., and Hayes, D. E., *Science*, **157**, 185 (1967).
- ³ Larson, R. L., and Pitman, W. C., III, *Bull. geol. Soc. Am.*, **83**, 3645-3662 (1972).
- ⁴ Vogt, P. R., Anderson, C. N., and Bracy, D. R., *J. geophys. Res.*, **76**, 4795-4823 (1971).
- ⁵ Taylor, P. T., Zietz, I., and Dennis, L. S., *Geophysics*, **33**, 755-780 (1968).
- ⁶ Vogt, P. R., Anderson, C. N., Bracy, D. R., and Schneider, E. D., *J. geophys. Res.*, **75**, 3955-3966 (1970).
- ⁷ Pitman, W. C., III, and Talwani, M., *Bull. geol. Soc. Am.*, **83**, 619-645 (1972).
- ⁸ Keen, C. E., Barrett, D. L., Manchester, K. S., and Ross, D. I., *Can. J. Earth Sci.*, **9**, 239-256 (1972).
- ⁹ IAGA Commission 2, Working Group 4, *J. geophys. Res.*, **74**, 4407-4408 (1969).
- ¹⁰ Irving, E., Robertson, W. A., and Aumento, F., *Can. J. Earth Sci.*, **1**, 226-238 (1969).
- ¹¹ Van Voorhis, G. D., and Walczak, J. E., *Informal Manuscript Report, No. M-8-63* (US Naval Oceanographic Office, 1963).

Fig. 4 Simplified contour charts of the magnetic anomaly field as observed (a), calculated from the Jurassic model (b) and the Triassic model (c). Anomalies greater than 20 gamma are shown in the dotted areas and anomalies less than -20 gamma are shown by dashed areas. For the Jurassic model a magnetic inclination of 60° and declination of 0° were used. For the Triassic model the values were 20° and 20° respectively. The remanent magnetisation was 1.1×10^{-2} e.m.u. cm^{-3} for both models. 1 km zones of reversed polarity and the same magnetisation were used in the models at the locations indicated by arrows.



Anomalous delays of teleseismic P waves in Yellowstone National Park

TELESEISMIC P waves recorded by a short-period seismic network, comprising 12 stations, in Yellowstone National Park, show anomalous delays of 1-2 s in their travel times in the central region of the park relative to the surrounding area. To explain this phenomenon, I propose that a substantial body of low velocity material is present beneath the park, with horizontal dimensions of several tens of kilometres; it may be the magma chamber associated with the volcanism of Yellowstone (ref. 1, and G. P. Eaton *et al.*, unpublished).

This study is based on records of about 65 teleseisms, distributed in a narrow range of azimuths north, south-east, west-south-west, and north-west from Yellowstone. Distances to the events range from 25° to 90°. About 25 events are available from the south-east and north-west azimuths and 15 from the other two combined. Because of operational problems the number of events used in this study varies from station to station.

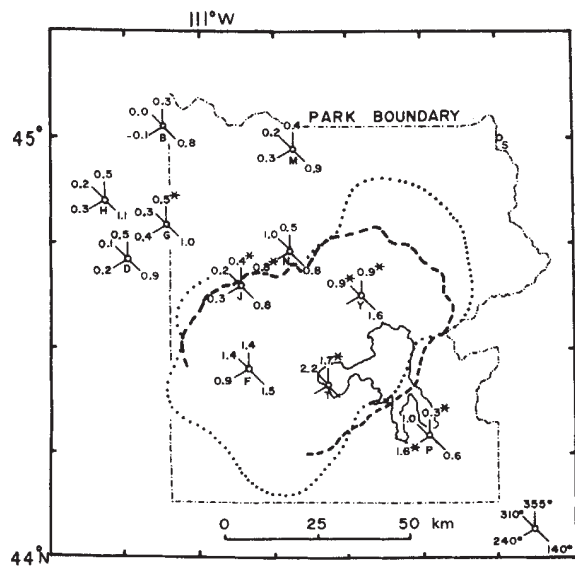


Fig. 1 Relative residuals in Yellowstone. Lettered dots, seismic stations; numbers, relative residuals with respect to station S, for events along the four azimuths (represented by the short lines); asterisks, uncertain values based on few available readings; dashed line, caldera boundary, inferred from geology and topography (ref. 1, and G. P. Eaton, unpublished); dotted line, top of steep gravity gradient^{7,8}

Travel-time residuals have been computed using the Seismological Tables for P by Herrin². These residuals contain a combination of station, path, and source effects and errors in earthquake location caused by the statistical nature of estimating hypocentres and origin times. Anomalous teleseismic residuals have been used in the past to study inhomogeneities in the physical properties of the Earth in the source regions of the earthquake³, along propagation paths of seismic waves⁴ and under recording stations^{5,6}. In order to isolate source and path effects from the effect of any anomalous structure in the immediate vicinity of the seismic network, one station is chosen as a reference. Residuals at the reference station are subtracted from the residuals at the other stations for each event and so-called relative residuals are obtained. Their variation across a network reflects the variation of the underlying crust and upper mantle.

The correct choice of reference station is critical; ideally it should be at a site which represents the average regional structure and which has no local anomalies. Geological evidence suggests that one of the stations well outside the Yellowstone caldera (ref. 1, and G. P. Eaton *et al.*, unpublished) is a good choice (Fig. 1). To make this selection more rigorous, average residuals for events in the north-western and south-eastern azimuths have been computed at six stations at which more than 10 events are available for

each direction (Table 1). For the north-west, the residuals at the five stations outside the caldera are in the range 2.4–2.7 s but are about 1.5 s higher at station F (Fig. 1), inside the caldera. For the south-eastern azimuth the residuals are 3.0–3.4 s at stations north-west of the caldera, and 4.0 s at station F. Thus, it seems that waves from the south-east, which pass under the Yellowstone caldera at some depth, are delayed relative to waves travelling from the north-west. Only at station S (Fig. 1) is the average residual for both azimuths comparable to the residuals for waves that do not travel under the caldera. It therefore seems reasonable to assume that the average residuals at station S are representative of regional structure and independent of sub-caldera structures; so station S is used as the reference.

Relative residuals with respect to station S at four typical stations, for events in the north-westerly and south-easterly directions, plotted as a function of distance from the event, show clearly the azimuthally dependent travel-time delays in Yellowstone (Fig. 2). At station F the relative residuals remain more or less constant at around 1.5 s for both azimuths, in the available distance range of 25°–85°; at stations G, M and B, on the other hand, the south-eastern residuals are consistently higher than the north-western values by about 0.8 s, suggesting the presence of a low velocity body under the caldera. There is some inevitable scatter in the plots, with a standard deviation of about 0.2 s around the average values. Of these stations, only station B shows a significant distance effect: the residuals around 80° are smaller than those at shorter distances by about 0.5 s. The angle of emergence of the rays changes from

Fig. 2 Variation of relative residuals as a function of distance at stations F, G, M and B. Dots and crosses, events in the south-eastern and north-western azimuths, respectively; solid and dashed lines, average of the data for those directions.

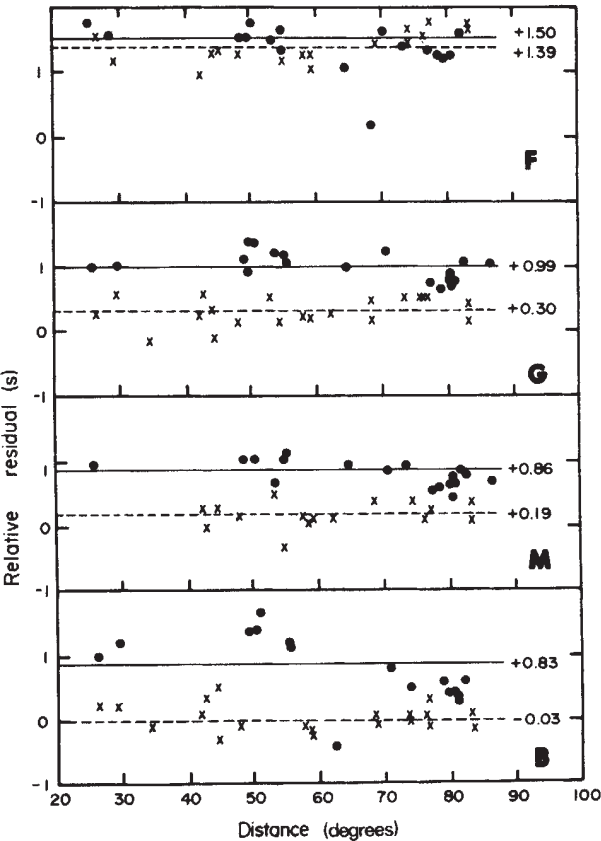


Table 1 Average residuals R , for events in the south-eastern and north-western azimuths

Station	South-east				North-west		
	No. of events	R (s)	Standard deviation		No. of events	R (s)	Standard deviation
M	23	3.3	0.6		17	2.7	0.5
F	19	4.0	0.5		19	3.9	0.4
G	22	3.4	0.6		23	2.7	0.5
D	14	3.0	0.6		17	2.7	0.5
B	18	3.2	0.7		23	2.4	0.5
S	22	2.3	0.6		23	2.5	0.6

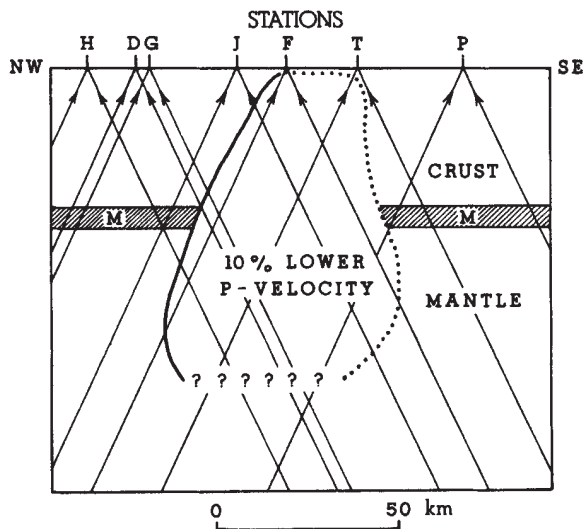


Fig. 3 North-westerly trending vertical cross section of the low velocity body that possibly explains the travel time delay pattern. The boundary of the body is shown by a heavy solid line where well constrained by the data, by a dotted line where only poorly constrained, and by question marks where the boundary cannot be determined. Arrows, schematic ray paths for events in the north-western and south-eastern azimuths. Width of the dark shaded band (Moho) indicates uncertainty in crustal thickness¹³.

30° to 17° to the vertical as distance changes from 25° to 80°. The distance effect could occur if a low velocity body, of a particular shape and position provides equal path lengths for rays that reach stations F, G and M at various angles, while at station B, the steeper rays sample a deeper and probably narrower part of the body.

Consideration of the average relative residuals at stations F, Y and T within the caldera (Fig. 1), shows that station F records large delays for all azimuths. Although the quality and quantity of data at stations Y and T are poor, the good data and a careful examination of all the available low quality seismograms show that stations Y and T are similar to station F, with high delays for all azimuths. Station N, at the edge of the caldera, almost fits into this pattern, except that it records a slightly lower northerly delay. As a first-order interpretation of the delay pattern at stations F, N, Y and T, it can be postulated a body with lower compressional wave velocity than the surrounding rocks exists under the central region of Yellowstone National Park. The top of the body must be close to the surface in the middle of the park, as shown by the large delays for all azimuths. At the north-western group of stations, M, B, G, H and D, which is outside the caldera, the relative residuals are about 1 s for events from the south-east and much lower for the other azimuths. The low velocity material must, therefore, extend to a depth such that the rays from the south-east reaching the north-western group of stations at angles of 17°–30° to the vertical traverse through that material.

It is also possible roughly to define the horizontal extent of the low velocity body. There is a sharp difference in the azimuthal pattern of relative residuals between stations F and J (Fig. 2). The delays are high for all azimuths at station F, whereas at station J, the south-easterly delay is much higher than for the other azimuths, indicating the existence of a boundary to the body to the south-east at some depth from station J. The northerly delays decrease from the high values at stations F, T and Y to about 0.5 s

at stations J and N, indicating shorter paths through the body for rays reaching the latter stations; that indicates a boundary not far north of the caldera boundary. South-easterly delays seem to decrease between stations Y and P, placing a limit on the south-easterly extent of the body. The available data are not inconsistent with the *ad hoc* hypothesis that the horizontal boundary of the top of the body is near the caldera rim.

The region of the Yellowstone caldera has a negative Bouguer gravity anomaly with sharp gradients close to the geologically determined caldera boundary^{7,8} (Fig. 1). The gravity low is believed to be produced by the partial presence of low density material such as rhyolite flows, magma and caldera fill. That explains only a small fraction of any observed teleseismic delays, however: a 10 km thickness of material with a conducting seismic velocity 10% lower than the surrounding rock would introduce a delay of only 0.2 s. Only teleseismic evidence, in particular the large delays at the north-western group of stations, shows that seismic rays are delayed at some depth under the caldera. High frequency seismic waves from local earthquakes in the region are severely attenuated as they cross the Yellowstone caldera (G. P. Eaton *et al.*, unpublished). Yellowstone has a history of volcanism extending from about 2 Myr ago to as recently as 0.07 Myr ago. A low velocity body comprising rhyolitic magma a few kilometres thick overlying an immense volume of basaltic magma and thermally disturbed crustal and mantle rocks, could be responsible for the gravity anomaly, the seismic wave attenuation, and the teleseismic delays; it is also consistent with the geological evidence (ref. 1, and G. P. Eaton, unpublished).

A tentative model for the low velocity body is suggested. If the compressional velocity in this body is assumed to be 10% lower than in the surrounding granite, the pattern of delays observed along a section from stations P to H are explicable (Fig. 3). This is a very conservative model based on an angle of emergence at the surface of 25°, for events at an average distance of about 50° from Yellowstone.

It has been proposed that Yellowstone lies above a deep mantle 'plume' of hot magma, perhaps 100–200 km in diameter, rising from the lower mantle^{10,11}. Anomalous seismic velocities, recorded using teleseismic methods, have been reported near the core-mantle boundary under Hawaii, another likely 'plume' region^{4,12,13}. It is of interest that even at the most north-western station, B, large south-easterly delays still persist, indicating that the anomalous body is perhaps deeper than the maximum depth seen by the network. Preliminary results, obtained using a north-westerly profile of portable stations extending about 200 km from the boundary of Yellowstone Park, show that the anomalous body may extend several hundred kilometres deep into the upper mantle¹⁴.

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- Christiansen, R. L., and Blank, H. R., Jr., *Prof. Pap. US geol. Surv.*, **729-B** (1972).
- Herrin, E., *Bull. seism. Soc. Am.*, **58**, 1193 (1968).
- Jacob, Klaus H., *J. geophys. Res.*, **77**, 2556 (1972).
- Julian, B. R., and Sengupta, M. K., *Nature*, **242**, 443 (1973).
- Cleary, J., and Hales, A. L., *Bull. seism. Soc. Am.*, **56**, 467 (1966).
- Iyer, H. M., and Healy, J. H., *J. geophys. Res.*, **77**, 1503 (1972).
- Pakiser, L. C., and Baldwin, L., Jr., *Prof. Pap. US geol. Surv.*, **424-B**, (1961).
- Blank, H. R., Jr., and Gettings, M. E., *US Geological Survey Open-file Map* (1974).
- Meyer, R. P., Steinhart, J. S., and Bonini, W. E., *Publs Carnegie Inst.*, **622**, 305 (1961).
- Morgan, W. J., *Mem. Am. Geol. Soc.*, **132**, 7 (1972).
- Mathews, V., and Anderson, C. E., *Nature*, **243**, 158 (1972).
- Davies, D., and Sheppard, R. M., *Nature*, **239**, 318 (1972).
- Kanasewich, E. R., Ellis, R. M., Chapman, C. H., and Gutowski, P. R., *J. geophys. Res.*, **78**, 1361 (1973).
- Iyer, H. M., Evans, J. R., and Coakley, J., *Eos, Trans. am. geophys. Un.*, **56**, 1190 (1974).

Duration of the Kuroko mineralisation episode

THE Kuroko strata-bound sulphide deposits in Japan, which are mined for lead, zinc, copper, silver and gold, are apparently related to Middle Miocene submarine acidic volcanism of the Green Tuff region¹⁻⁵ (Fig. 1). Most of the Kuroko deposits occur in the upper part of the Nishikurosawa stage (≈ 14 Myr) of the Middle Miocene and, on geological grounds, are considered to have been formed in a short time⁶. Here I describe an attempt to determine the length of time involved from the magnetic polarity of associated rocks.

Most of the results have been obtained from red ferruginous chert, which commonly overlies the Kuroko ore in beds 1–10 m thick. Chert deposition seems to have been the last stage in the Kuroko mineralisation process⁷. The chert consists of quartz (diameter 10–600 μm) and very fine grained haematite, with minor pyrite and barite. The average grain sizes^{8,9} of the haematite determined from Furutobe and Kosaka mines (Fig. 1) are 150 $\text{\AA}$ and 95 $\text{\AA}$ respectively. The initial grain size between the single-domain and superparamagnetic range is usually assumed to be about 290 $\text{\AA}$ (ref. 10), so much of the haematite is probably superparamagnetic. Nevertheless a substantial number of larger grains must be present because the remanent magnetisation is very stable. The remanent intensity drops only a few per cent, and the directions remain unchanged, after demagnetisation in alternating fields of 600 oersted. Also hysteresis loops for fields up to 19,000 oersted show essentially a straight-line increase, with no tendency to saturation. The magnetisation is probably a chemical remanent magnetisation^{11,12}.

Orientated samples of cherts and other rocks were collected from the Hokuroku and Aizu areas (Fig. 1). The Hokuroku area contains the greatest concentration of Kuroko deposits and all mines have similar geological sections, except that basalt (B_1) occurs at Furutobe (Fig. 2). Two marker mudstone beds (M_2 and M_1) allow the ore horizons to be completed

Fig. 1 Distribution of Kuroko deposits. *a*, Zone of Miocene volcanism; *b*, zone of Neogene folding.

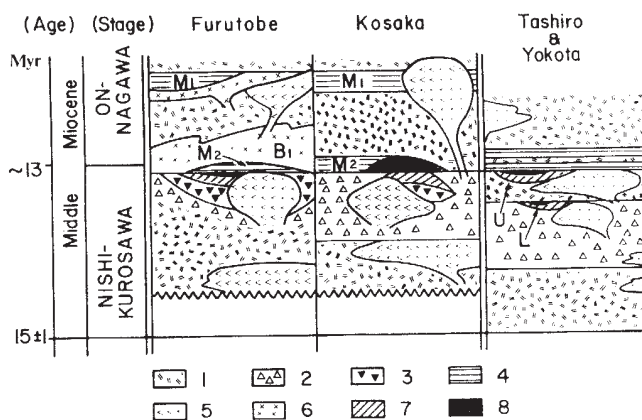
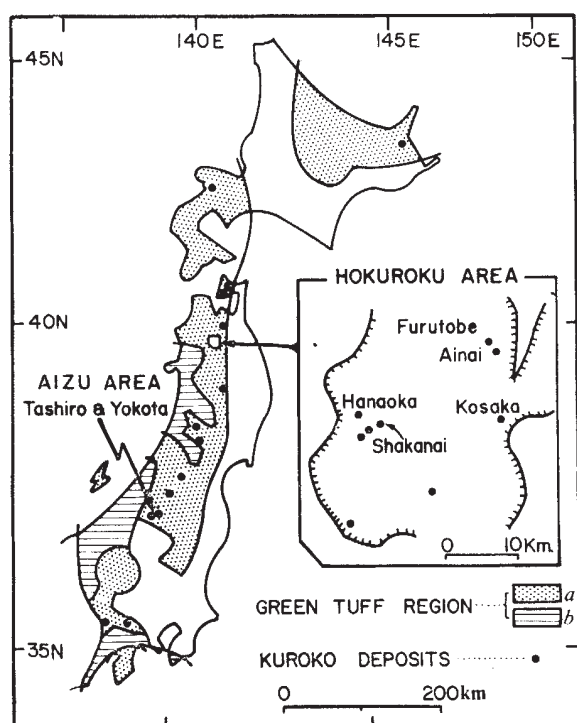


Fig. 2 Geological columns of the Furutobe and Kosaka mining districts in the Hokuroku area, and of the Tashiro and Yokota mining districts in the Aizu area. 1, Tuft; 2, tuft breccia; 3, volcanic breccia; 4, mudstone; 5, rhyolite; 6, basalt; 7, Kuroko ore body; 8, ferruginous chert. L, Lower ore bodies; U, Upper ore bodies. For explanation of M_1 , M_2 and B see text. The ages are from Ikebe *et al.*²¹.

(Fig. 2). The lower half of M_2 has microfossils of the Nishikurosawa stage and its upper half has microfossils of the On-nagawa stage^{3,13}. At Furutobe mine in the Hokuroku area the sequence is Kuroko ore, ferruginous chert, mudstone M_2 and basalt B_1 (ref. 14) (Fig. 3). The chert has reversed magnetisation (10 samples, mean intensity 500 μgauss), the mudstone has normal magnetisation (three samples, intensity 50 μgauss , remanent coercivity 200 oersted) and the basalt has normal magnetisation (three samples, intensity 2,000 μgauss , remanent coercivity 150 oersted). At Aina mine¹⁵ the ferruginous chert has reversed magnetisation (five samples, intensity 50 μgauss). The chert at Hanaoka mine has both normal and reversed magnetisation (two normal samples, six reversed samples, intensity 30 μgauss and at Shakanai mine⁵ the ferruginous chert that occurs in the lower part of the ore body has reversed magnetisation (three samples, intensity 200 μgauss). The ferruginous chert at Kosaka mine occurs in the mudstone M_2 (ref. 16) (Fig. 3). Samples taken from directly above, and at some distance from, Kuroko ore have normal magnetisation (nine samples, intensity 100–800 μgauss).

The Tashiro and Yokota mines in the Aizu area have similar geological sequences¹⁷. There are two mudstone layers; the lower contains microfossils of the Nishikurosawa stage, and the upper microfossils of the On-nagawa stage, just as in the Hokuroku area (Fig. 2). The Aizu area is exceptional in that there are two different ore horizons—there is an upper ore body at the same horizon as in the Hokuroku area and a lower one (Fig. 2). Two layers of chert directly overlay both upper and lower ore bodies. Ferruginous chert associated with the upper ore bodies has reversed magnetisation (six samples, intensity 50 μgauss) but that associated with the lower ore bodies has normal magnetisation (six samples, 50 μgauss).

The polarity results are plotted on the basis of the known stratigraphy in Fig. 3. All the Kuroko deposits, except the lower ore bodies in the Aizu area, seem to have been formed in one reversed polarity interval. Soon after the formation of the Kuroko deposits the geomagnetic field changed from reversed to normal, and lower ore bodies in the Aizu area were apparently formed in the preceding normal interval. The upper part of the Nishikurosawa stage seems, however, to have been laid down in an interval of reversed polarity. This is consistent with evidence of the short reversal interval in the upper part of the Nishikurosawa stage from measurements on volcanic and sedimentary rocks in the Green Tuff region^{18,19}.

If the magnetic stratigraphy of the Middle Miocene were perfectly known, the exact time interval during which the

Kuroko deposits formed could be determined from the evidence summarised in Fig. 3. Of course this is not yet possible, but an estimate can be made from the time scale of Heirtzler *et al.*²⁰. On this scale five reversed intervals occur between 12 and 15 Myr ago and they have an average duration of 0.2 Myr. As the Kuroko deposits, apart from a few exceptional deposits which occur in a different horizon, were formed in a single polarity interval within that time span, it is reasonable to conclude that they were laid down in less than 0.2 Myr. These results support the supposition that the Kuroko ores are of syngenetic origin¹⁻⁷. They also indicate that the ores were deposited essentially contemporaneously in both the Aizu and Hokuroku areas, more than 300 km apart.

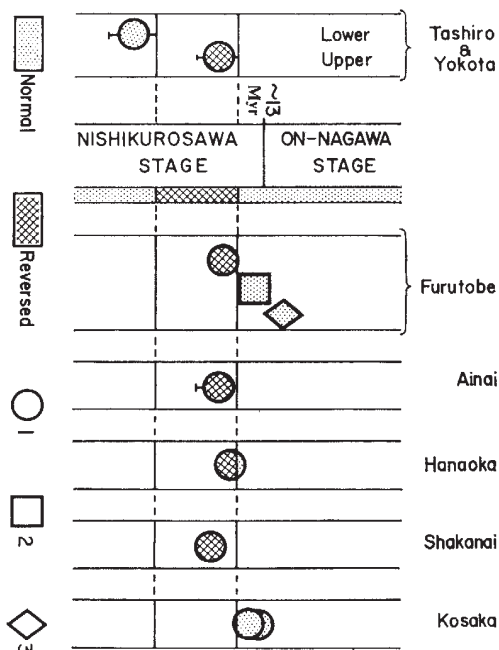


Fig. 3 Polarities plotted on the basis of the known stratigraphic sequence. 1, Ferruginous chert; 2, mudstone; 3, basalt. Upper and Lower are the upper and lower ore bodies in the Aizu area.

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- 1 Horikoshi, E., *Min. Deposita*, **4**, 321-345 (1969).
- 2 Tatsumi, T., Sekine, Y., and Kanehira, K., in *Volcanism and Ore Genesis* (edit. by Tatsumi, T.), 3-47 (University of Tokyo Press, Tokyo, 1970).
- 3 Matsukuma, T., and Horikoshi, E., in *Volcanism and Ore Genesis* (edit. by Tatsumi, T.), 153-179 (University of Tokyo Press, Tokyo, 1970).
- 4 Horikoshi, E., and Sato, T., in *Volcanism and Ore Genesis* (edit. by Tatsumi, T.), 181-195 (University of Tokyo Press, Tokyo, 1970).
- 5 Kajiura, Y., in *Volcanism and Ore Genesis* (edit. by Tatsumi, T.), 197-206 (University of Tokyo Press, Tokyo, 1970).
- 6 Sato, T., *Min. Geol., Tokyo, Special Issue*, **6** (in the press).
- 7 Sato, T., *Min. Geol., Tokyo*, **18**, 241-256 (1968). (In Japanese).
- 8 Stokes, A. R., *Proc. phys. Soc. Lond.*, **61**, 382-391 (1948).
- 9 Warren, B. E., and Averbach, B. L., *J. appl. Phys.*, **21**, 595-599 (1950).
- 10 Nagata, T., *Rock Magnetism* (Muruzen, Tokyo, 1961).
- 11 Haigh, G., *Phil. Mag.*, **3**, 267-286 (1958).
- 12 Kobayashi, K., *J. Geomagn. Geoelect. Kyoto*, **10**, 99-117 (1959).
- 13 Otagaki, T., *Min. Geol., Tokyo*, **16**, 237-248 (1966). (In Japanese).

- 14 Tanaka, T., and Lu, K. I., *Min. Geol., Tokyo*, **19**, 312-332 (1969). (In Japanese).
- 15 Ishikawa, Y., and Yanagizawa, Y., *Min. Geol., Tokyo*, **21**, 138-149 (1971). (In Japanese).
- 16 Suzuki, Y., Tanimura, S., and Hashiguchi, H., *Min. Geol., Tokyo*, **21**, 329-346 (1971). (In Japanese).
- 17 Shimada, I., and Hirabayashi, T., *Min. Geol., Tokyo*, **22**, 329-346 (1972). (In Japanese).
- 18 Noritomi, K., Sato, S., and Hayashi, K., *Res. Inst. Underground Resources, Akita Univ.*, **39**, 32-73 (1970). (In Japanese).
- 19 Ueno, H., *Econ. Geol.* (in the press).
- 20 Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., III, and Pichon, X. L., *J. geophys. Res.*, **73**, 2119-2136 (1968).
- 21 Ikebe, N., Takayanagi, Y., Chiji, M., and Chinzei, K., in *Pacific Geology No. 4* (edit. by Minato, M.), 39-78 (Tsukiji Shokan, Tokyo, 1972).

Deglaciation of the Labrador continental shelf

It has long been inferred from studies of glacial features on land that the North American Quaternary glaciers extended far out on the Atlantic continental shelf^{1,2}. Only within the last ten years, however, have offshore studies^{3,4} on the Scotian Shelf convincingly demonstrated the presence of a system of submarine moraines. This study, which used 1,100 km of deep-towed 3.5 kHz sparker profiles, 1,200 km of side-scan sonar records, 2,000 km of echo sounding profiles and 165 bottom sediment samples collected during cruises of CSS Dawson and CFAV Sackville has identified moraines and other relict glacial land forms on the outer Labrador Shelf.

More than 20% of the Hamilton Bank area is topographically rough (bottom type I, Fig. 1) and has a local relief of 10-30 m (Fig. 2c, d). Side-scan sonar representations of this irregular surface are characterised by mottles; bathymetric charts⁵ show numerous roughly circular, closed depressions deeper than 10 m. Adjacent areas with a more gently undulating surface without closed bathymetric depressions are recognised as a separate morphological region (bottom type II, Fig. 1). A zone of extremely smooth, flat topography (bottom type III, Fig. 1) is distinguished as still another morphological unit. Side-scan records reveal that the uniform bottom in this area is relieved only by occasional iceberg plow marks. Sparker profiles reveal a hard bottom with acoustic penetration generally <10 m, similar to the rest of the bank.

Moraines of the Hamilton Bank region (Fig. 2a, b) seem identical in dimensions and in transverse section to those of the Scotian Shelf³ and to terrestrial end moraines in southern New England². Moraines are traceable along the foot of the landward facing bank margin and across the surface of the bank (Fig. 1).

The sharpness of existing relict features implies that they have been affected little by post-glacial sedimentary and erosional processes. Surface sediments, however, are not relict and seem to have reached a partial equilibrium with present oceanographic conditions. In general, the sediments are similar to those reported from elsewhere in glaciated offshore eastern Canada and New England^{3,6-10}.

Identification of probable end moraines and the ubiquitous distribution of coarse gravel and cobbles, as well as the excellent state of preservation of glacial landforms, strongly imply that the entire continental shelf was glaciated by the Laurentide ice sheet during the Wisconsin. Coarse sediments, which appear in places on the continental slope to at least a depth of 400 m, may have been deposited beneath a floating ice shelf¹¹. Sills covered by a coarse gravel and boulder lag at the seaward end of Cartwright and Hawke Channels are interpreted to be mounds of detritus deposited beneath the ice shelf at a point just seaward of the grounding line¹². Although Hamilton Bank was completely ice covered, the greatest ice discharge seems to have been through these transverse channels. There is on average about 100-150 m of glacial drift in the channels compared with 50 m on the bank^{13,14}.

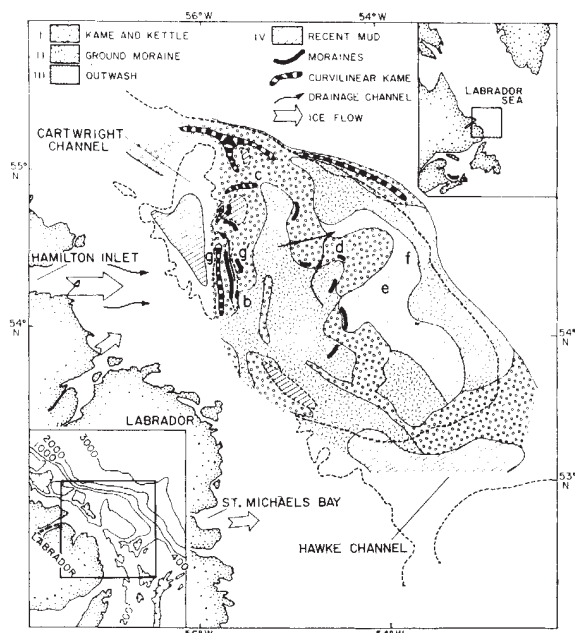


Fig. 1 Physiographic-surficial geology map of the Hamilton Bank area. The dashed line is the 300 m contour and all areas completely enclosed by this contour are basins. Letters refer to sections in Fig. 2. Flow directions of continental ice are inferred from the Glacial Map of Canada²⁰.

Once deglaciation had begun, ice on the bank must have retreated by calving until an equilibrium between ice thickness and water depth was reached^{15,16}. Bottom type I (Fig. 1) seems to have formed as the remaining portion of the grounded ice sheet melted *in situ*. Morphologically, this bottom closely resembles terrestrial kame and kettle topography. This, together with the stratification detected in cores¹⁰, leads me to term these features 'subaqueous' kames and kettles. Kames probably formed beneath the stranded, stagnant ice sheet (Fig. 3) as cavities caused by uneven bottom melting filled with an accumulation of semi-stratified detritus dropped from the undersurface of the ice¹¹. Subaqueous kettle holes seem to be the result of the disintegration and flotation of grounded masses of ice that had been surrounded by drift.

Melt water flow and perhaps tidal currents beneath the grounded ice formed a system of drainage channels (Fig. 1) and provided a route for the transport of bed load and suspended sediment by hyperpycnal flow to deglaciated portions of the shelf. The resulting deposits, winnowed and spread southward over previously deposited drift by the prevailing Labrador Current, may have created bottom type III (Fig. 1). This area is as flat as (Fig. 2e, f), and seems to be analogous to, terrestrial outwash plains such as those of New England on Marthas Vineyard and Long Island².

Curvilinear kame features (Fig. 1) are recognised by their relatively smooth flat tops and weak stratification with some evidence of ice contact disruption of bedding near the margins (Fig. 2g-g'). They are thought to have formed where debris laden glacial runoff was impounded in temporary ice lakes or bays situated between ice tongues. The transverse channel sills and a terrace along the seaward edge of the bank (Fig. 1), although generated beneath a floating ice shelf, are included in this category because it seems probable that they consist of semi-stratified drift. In contrast, end moraines on the bank are believed to have formed along a grounded ice sheet margin. Their irregular topography is seen as indicative of rapid deposition with little chance for the development of stratification. The

curvilinear trend of end moraines across the central bank (Fig. 1) was probably formed during a readvance of the bank ice cover following the initiation of general deglaciation. The gently undulating surface (bottom type II, Fig. 1) which may be ground moraine, marks the area overridden by this readvance.

Preserved ground moraine and a lack of subaqueous kames and kettles landward of the end moraine complex indicate that once the readvance had ceased, the ice sheet broke up and dispersed with little melting in place. For the remainder of the deglaciation period, active ice tongues and stagnant ice lobes were confined to the transverse channels where they gradually decreased in size leaving a complex pattern of marginal and end moraines, kame terraces and kame and kettle topography.

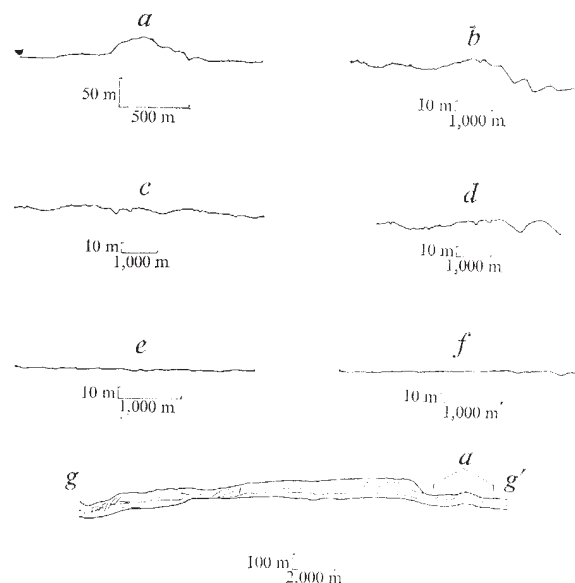


Fig. 2 Traced profiles of glacial and pro-glacial features on Hamilton Bank: a, b, moraines; c, d, kame and kettle topography; e, f, outwash plain; g-g', large linear kame, with weak stratification evident. Types of profiles include echo sounding (a), deep-towed sparker (b, c, d, e, f) and 5 inch³ air gun interpretation (g-g', ref. 29).

It is significant that Wisconsin glacial ice was grounded on Hamilton Bank and in the transverse channels, because it has been established that at water depths greater than a critical value, buoyancy and the strength of glacial ice permit the survival of only those ice sheets that have sufficient lateral resistance to spreading¹⁵⁻¹⁸. This resistance may be supplied by confinement between anchoring head-lines or by a strong coupling to the substrate as in a cold (frozen) based ice sheet¹⁵. In West Antarctica the critical depth is about 170 m (ref. 17). Allowing for a Wisconsin glacioeustatic sea level lowering of 100 m (ref. 19), 80 m or more of glacioisostatic depression on the bank, which today averages about 190 m below sea level, would have produced depths greater than 170 m. Because the levels of raised beaches and marine deposits along the south-eastern Labrador coast²⁰ make at least that amount of glacioisostatic depression extremely likely, an initially cold based ice sheet on the bank is strongly implied. Thus, deglaciation of the Labrador Shelf in the Hamilton Bank area may have been directly caused by a decreased resistance to spreading by decoupling the ice from the substrate. This occurred

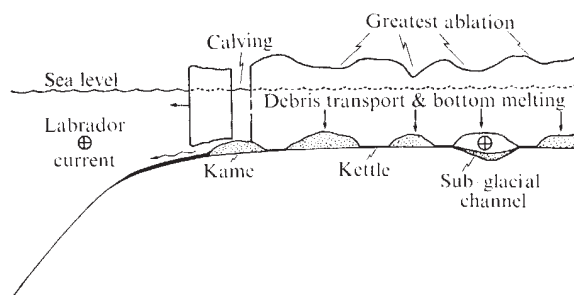


Fig. 3 Schematic representation of hummocky stagnant ice sheet on Hamilton Bank. Stippling represents semi-stratified drift and channel deposits. Black shading is outwash transported by hyperpycnal outflow (wavy arrow) from subglacial channels. This may have been augmented by tidal currents. Debris freed from the ice sheet by bottom melting is shown accumulating on ice free portions of the bottom forming subaqueous kame deposits. Subaqueous kettles are produced by the flotation and removal of grounded ice masses.

probably during a period of rising summer air temperatures which warmed the ice sheet and precipitated a change from cold to wet based glacial conditions. In Antarctica grounded ice sheets and ice shelves are thought to be unstable where the average air temperature for the warmest month is above 0°C (ref. 16).

Faunal evidence indicates a warming of ocean water in the eastern Arctic region beginning before 8,000 yr BP (refs 21, 22). The Cockburn-Cockrane readvance of closely similar age has been interpreted²⁴⁻²⁵ to be the result of a glacial surge to a new equilibrium profile in response to rapid deglaciation of a part of Hudson Bay²³. It is therefore tempting to speculate that the retreat of ice from the seaward edge of Hamilton Bank occurred at about the same time as the opening of Hudson Bay and that both areas were deglaciated in response to a general warming of the ice sheet. The ensuing readvance on Hamilton Bank is seen as an adjustment to a new equilibrium profile and as a corollary to the Cockburn-Cockrane event²³.

A radiometric deglacial chronology for south-eastern Labrador has not yet been established. Deglaciation dates of 16,000–14,000 yr BP have been inferred for the Labrador Shelf²³, but published²⁶ and unpublished ¹⁴C dates from the Hamlet Inlet area are consistently younger than 8,700 yr BP. These dates and the probable triggering effect of the climatic warming imply that deglaciation of the Labrador Shelf may have been in progress as late as 9,000 yr BP (refs 26–28).

Eastcan Exploration Ltd, Calgary, supplied the V-fin used in this study. Information on unpublished ¹⁴C dates was supplied by R. J. Fulton of the Geological Survey of Canada. Dr W. J. M. van der Linden proposed this project and collected most of the data.

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- 1 Goldthwait, J. W., *Geol. Surv. Can. Mem.*, **140**, (1924).
- 2 Woodworth, J. B., Wigglesworth, E., et al., *Harvard Univ. Mus. Comp. Zool. Mem.*, **52**, (1934).
- 3 King, L. H., *Geol. Soc. Am. Bull.*, **80**, 83–96 (1969).
- 4 King, L. H., MacLean, B., and Drapeau, G., *24th Int. Geol. Congr., Canada*, **8**, 237–249 (1972).
- 5 Monahan, D., *Bathymetric Charts 18632, -34, -42, -44, -46, -54, -56*, (Natural Resour. Chart Series, Can. Hydrogr. Surv., (in the press).
- 6 Slatt, R. M., *Can. J. Earth Sci.*, **11**, 362–368 (1974).
- 7 Slatt, R. M., *Geol. Soc. Am. Bull.*, **85**, 821–826 (1974).
- 8 King, L. H., *Can. Dept. Energy, Mines and Resources, Mar. Sci. Branch*, (1970).
- 9 Garrison, L. E., and McMaster, R. L., *Mar. Geol.*, **4**, 273–289 (1966).
- 10 Slatt, R. M., and Lew, A. B., *J. sediment. Petrol.*, **43**, 1054–1060 (1973).
- 11 Fillon, R. H., *Nature phys. Sci.*, **238**, 40–42 (1972).
- 12 Barnett, D. M., and Holdsworth, G., *Can. J. Earth Sci.*, **11**, 380–408 (1974).

- 13 Grant, A. C., thesis, Dalhousie Univ. (1971).
- 14 Grant, A. C., *Can. J. Earth Sci.*, **9**, 1394–1430 (1972).
- 15 Weertman, J., *J. Glaciol.*, **13**, 3–11 (1974).
- 16 Mercer, J. H., *Pub. Int. Ass. Sci. Hydrol.*, **79**, 217–225 (1968).
- 17 Hollin, J., *J. Glaciol.*, **4**, 173–195 (1962).
- 18 Robin, G. de Q., and Adie, R. J., *Antarctic Research*, (edit. by Priestly, R., Adie, R. J., and Robin, G. de Q.), ch. 8 (Butterworth, London 1964).
- 19 Fairbridge, R. W., in *Physics and Chemistry of the Earth*, (edit. by Ahrens, L. H., Press, F., Rankama, K., and Runcorn, S.K.), ch. 3, (Pergamon, 1961).
- 20 Prest, V. K., Grant, D. R., and Rampton, V. N., *Glacial Map of Canada*, (Can. Geol. Surv., Ottawa, 1967).
- 21 Andrews, J. T., *Palaeogeogr., Palaeoclimatol., Palaeoecol.*, **11**, 157–176 (1972).
- 22 Andrews, J. T., Mears, A., Miller, G. H., and Pheasant, D. R., *Nature phys. Sci.*, **239**, 147–149 (1972).
- 23 Prest, V. K., in *Geology and Economic Minerals of Canada* (edit. by Douglas, R. J. W.), ch. 12 (Can. Geol. Surv., Ottawa, 1970).
- 24 Andrews, J. T., and Ives, J. D., in *Climatic changes in Arctic areas during the last ten-thousand years* (edit. by Vasari, I., Hyvarinen, H., and Hicks, S.), 149–171, (Univ. Oulu, Finland, 1972).
- 25 Bryson, R. A., Wendland, W. M., Ives, J. D., and Andrews, J. T., *Arctic and Alpine Res.*, **1**, 1–14 (1969).
- 26 Hodgson, D. A., and Fulton, R. J., *Paper 72-1B*, 102–105 (Can. Geol. Surv., 1972).
- 27 Wenner, C. G., *Geogr. Ann.*, Arg. XXIX, Häft. 3–4, 137–373 (1947).
- 28 Blake, W., Jr., *Geogr. Bull.*, No. 9, 75–100 (1956).
- 29 Vilks, G., Rashid, M. A., and van der Linden, W. J. M., *Can. J. Earth Sci.*, **11**, 1427–1434 (1974).

Survey of three megalithic sites in Argyllshire

THREE sites showing simple alignments of standing stones in Argyllshire were tested for possible astronomical significance by members of the Cambridge University Astronomical Society during August and September 1973. The sites were surveyed in detail with a portable (20" circles) vernier theodolite, on which comprehensive tests were made before and after the expedition to determine adjustment errors; brief checks were made on site. Absolute azimuths were determined accurately by making timed observations of the Sun using MSF Rugby as the standard time signal. The error in azimuth was normally $\pm 10''$ and in altitude $\pm 20''$, although the latter value had to be modified to take account of uncertainties in atmospheric refraction. There is extensive information available on the Ballochroy site¹⁻³, and brief details of the sites at Loch Seil⁴⁻⁶ and Loch Nell^{4,6} have been published.

As so much importance has been attached to the site at Ballochroy we surveyed it hoping to produce more accurate profile measurements. The site contains three menhirs aligned with the Island of Cara to the south-west, with the flattened faces of the central stone indicating approximately the direction of Corra Bheinn on Jura to the north-west. A kist lies 40 m south-west of the three menhirs, and is

Fig. 1 Midwinter sunset over Cara Island (NR638438) shown for, a, 1600 BC (obliquity of ecliptic, $\epsilon = 23^{\circ}53'09''$) and, b, 1800 BC ($\epsilon = 23^{\circ}54'28''$) as seen from the menhirs or the kist at Ballochroy. Profile of Cara shown below. Errors in altitude of $\pm 0.7'$ are estimated from a temperature variation of $\pm 3^{\circ}\text{C}$ around 5°C at sunset, and from a measurement error of $\pm 0.35'$.

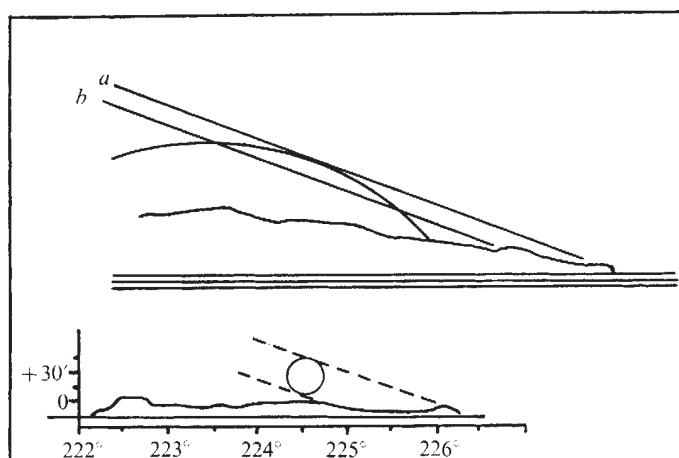


Table 1 Observed lines at Loch Seil (*a, b, c*) and Strontoiller (*d, e, f*)

Line	Az*	h*	δ^*	Notes
<i>a</i>			-21°	Alignment—no obvious foresight
<i>b</i>	326.2°	+5.3°	+32.1°	Indicated foresight
<i>c</i>			+6°	Alignment—no obvious foresight
<i>d</i>	305.3°	+3.6°	+20.9°	Cross sight line, notch
<i>e</i>	307.5°	+3.0°	+21.5°	Cross sight line, pit
<i>f</i>			-21.8°	Ref. 3 (not surveyed—trees)

*Azimuth (*Az*), altitude (*h*) and declination (δ) are given for well defined lines.

approximately collinear with them. Weather conditions during our survey were variable, especially over the Paps of Jura, but repeated observations over a 3-h period showed refraction to be constant. For calculating the track of the setting Sun we used astronomical refraction tables^{7,8}. We followed Thom's method⁹ to allow for changes in our measured altitudes caused by local terrestrial refraction.

An observer at the site looking towards Cara in 1580 (± 100) BC would have seen the top limb of the midwinter Sun set just clear of the island (Fig. 1). An observer looking towards the north-west from the central stone would have seen the midsummer Sun's upper limb clip the top of Corra Bheinn in 1640 (± 70) BC; standing near the kist in 1600 (± 70) BC he would have seen the Sun twinkle down the slope of Corra Bheinn (Fig. 2). Thom postulated^{1,3} that the site was constructed to observe these phenomena. The accuracy of our survey and the similarity between the dates quoted lends support to this view.

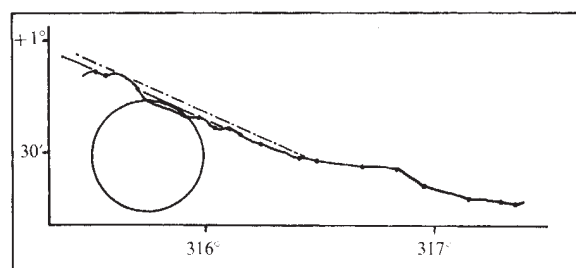


Fig. 2 Midsummer sunset behind Corra Bheinn (NR526755) at 1600 BC ($\epsilon=23^{\circ}53'09''$) as seen from centre menhir (---) and kist (—). Errors in profile altitudes of $\pm 0.5^{\circ}$ are estimated from a temperature variation of $\pm 3^{\circ}\text{C}$ around 13°C at sunset, and from a measurement error of $\pm 0.35'$.

The principal stones on the site near Loch Seil, Kilninver are three menhirs 2.5 m high; the centre stone is somewhat tilted. The stump of another menhir, apparently broken since Thom's survey⁵, was found 40 m away. The possible stump of another menhir, accurately in line with the other outlier and the centre menhir of the group of three, was found 180 m away. Skyline profiles, all of which were less than 3 km distant, were surveyed in both directions along the line of the three principal stones (Table 1, *a* and *b*), and in the direction indicated by the outliers (Table 1, *c*). Declinations for these lines are given in Table 1; we agree with Thom's declinations⁴ but find no well indicated foresight for his solar line and do not think his Capella line significant.

The site at Loch Nell, near Oban, is near a farm the name of which—Strontoiller—is Gaelic for 'point of light'. It has a menhir 4 m high and a possible 1 m menhir 50 m to the south-east with a ring cairn 3 m in diameter between them. The small menhir has a gate hung from it, but because of its size and shape it seems unlikely that it

was erected for this purpose. About 300 m to the north-west of the large menhir is a circle (15 m in diameter) of boulders, which is possibly all that remains of a despoiled cairn. An outcrop of rock, on which there seems to be a cairn 20 m in diameter, lies between the circle and the menhirs and restricts the view of the menhirs from the centre of the circle. The two menhirs are aligned with a small hill 600 m away.

The top of the larger menhir has been flattened (an unusual feature) and slopes at the same angle as the hill. The cross-sight lines indicated by opposite edges of the menhirs point accurately at two prominent gaps on the hillside (Fig. 3). One is a pit 4.5 m wide which has earthworks at its sides. The other is an apparently artificial, 1 m wide, V-shaped notch cut into the rock. The notch and pit

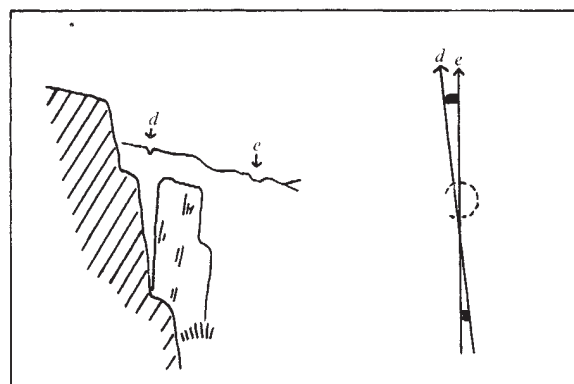


Fig. 3 Left, skyline profile to the west at Strontoiller seen from behind the 1 m menhir; 4 m menhir indicates notch *d*. Right, cross bearings indicate notches *d* and *e* with setting declinations $20^{\circ} 56.1'$ and $21^{\circ} 28.2'$. Ring cairn shown dotted.

lines (Table 1, *d* and *e*) indicate accurately a limb of the setting Sun at declinations $20^{\circ} 56.1'$ and $21^{\circ} 28.2'$ respectively. We think that these declinations have no particular astronomical significance. Thom⁴ gives another (calendar) line at this site (Table 1, *f*), indicated by the large menhir from the circle. The Ordnance Survey marks the farm on the hill as 'Duneil' (dun is Gaelic for fort), which suggests that the earthworks are of recent rather than megalithic origin. Also, there are further earthworks on the southern slope and behind the hill. Thus, we conclude that such convincing alignments may have occurred only by chance; this view, however, requires confirmation by independent archaeological work. (Other profiles indicated by the flat sides of the large menhir were surveyed but these too, in our opinion, have no astronomical significance.)

The survey of Ballochroy shows that the site could have been used to observe the midwinter and midsummer setting Sun in 1600 BC. The alignments at the other two sites are not, in our opinion, consistent with possible astronomical usage, despite the existence at the Loch Nell site of seemingly convincing artificial foresights.

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¹ Thom, A., *J. Br. astr. Ass.*, **64**, 402-3 (1954).

² Thom, A., *Megalithic Lunar Observatories*, 36, 37 (Oxford University Press, London, 1971).

³ Thom, A., *Megalithic Sites in Britain*, 151-154 (Oxford University Press, London, 1967).

⁴ *ibid.*, 97.

⁵ Thom, A., *Vistas Astr.*, **7**, 26 (1966).

⁶ *ibid.*, **7**, 9 (1966).

⁷ Thom, A., *Megalithic Lunar Observatories*, 34 (Oxford University Press, London, 1971).

⁸ Bessel's Refraction Tables, *Chambers Seven-Figure Mathematical Tables*, 430-431 (Chambers, Edinburgh, 1930).

⁹ Thom, A., *Megalithic Lunar Observatories*, 29, 30 (Oxford University Press, London, 1971).

Mineralogical alteration of Chinese tomb jades

ADMIRERS of ancient Chinese jades have frequently noted the curious white, chalky areas which invade the otherwise firm polished surfaces of many artefacts. This phenomenon, variously described as "alteration", "calcification", "calcination", or simply "decay", seems to be restricted to ancient (Shang to Han Dynasty, 1766 BC to 220 AD) 'tomb jades', artefacts explicitly or implicitly connected with burials¹. We have examined artefacts exhibiting this alteration using X-ray powder diffraction and scanning electron microscopy. We postulate that the alteration consists of a selective dissolution (leaching) along grain boundaries by ammoniacal solutions of high pH produced during decay of the corpses with which the jades were buried. Analyses by

Fig. 1 Chou Dynasty tiger (?) (Buffalo Museum of Science No. J-294) of pale green nephrite, illustrating typical partial alteration. Overall length 5.3 cm.

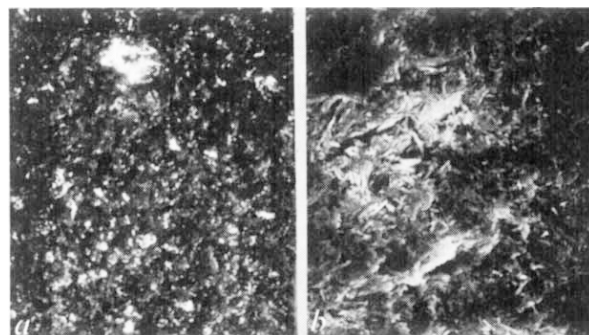
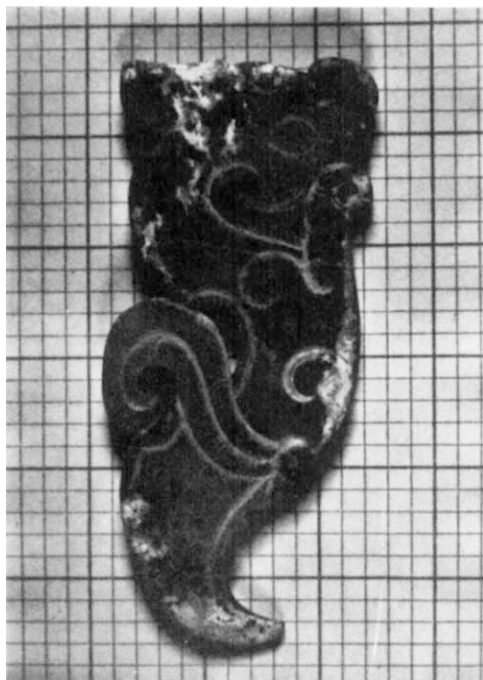


Fig. 2 Scanning electron photomicrographs of surface of nephrite artefact. *a*, Unaltered surface (width of field 100 μ m); *b*, altered surface (width of field 100 μ m).

X-ray powder diffraction of a number of partially altered jades showed them all to be nephrite (a fine grained, compact variety of the tremolite-actinolite series of amphiboles). This mineralogy is consistent with the antiquity of the artefacts, since the other jade mineral, jadeite (a pyroxene), was not introduced into China until the eighteenth century AD^{1,2}. Interestingly, the diffraction patterns of altered and unaltered regions are identical (also noted by West¹), indicating that the alteration does not involve any change in mineral composition. This invalidates the terms calcification and calcination which, respectively, imply chemical replacement and thermal decomposition. It is likely that these terms were coined with reference to the white, ivory-like 'chicken-bone' jades which are produced by heating nephrite: calcification because of the bone-like appearance, and calcination referring to the method of production. The original nephrite in the 'chicken-bone' jades has, however, decomposed with loss of structural water into a diopside-enstatite-silica assemblage which is easily distinguished in diffraction patterns.

Altered surfaces are characteristically white or buff-coloured; they are softer and less reflective than adjacent unaltered areas. Occasionally artefacts will be completely altered, usually with badly eroded surfaces, although more commonly, only certain areas are altered (Fig. 1), with only shallow penetration, preserving the details of intricately carved surfaces. The boundaries between altered and unaltered regions are typically relatively sharp. A curious patterned alteration occurs on some Archaic ceremonial knife blades, described as "fabric-marked", in the form of small orientated blebs, reminiscent of the pattern of a coarsely woven cloth.

The change in colour, hardness, and reflectivity of the altered surface, coupled with the lack of change in mineralogy, suggests chemical etching as a likely cause of the alteration. We therefore examined the surface microstructure of altered and unaltered areas of a Chou Dynasty (1122 to 206 BC) artefact (Fig. 1) with a scanning electron microscope. The results show that the unaltered surface is compact and smooth, even at high magnification (Fig. 2*a*), whereas the altered surface consists of an open, felted network of acicular tremolite crystals (Fig. 2*b*). (Comparative X-ray emission spectroscopy of altered and unaltered surfaces reveals no significant difference in chemical composition.)

This suggests that the mechanism of alteration is a selective congruent dissolution (leaching) along grain boundaries. It has previously been suggested that the alteration may result from the action of soil acids and moisture^{1,2}, and may be accelerated by decomposition products of the bodies in the tombs¹. Experimental studies³ indicate that the solubility of tremolite is at a minimum in solutions of pH from 3 to

8, the range of most natural soil waters. Furthermore, solubility studies of tremolite and other silicate minerals show that dissolution in acidic waters is incongruent, leaving a solid residue of silica, and is extremely slow⁴. Dissolution in basic ($pH \geq 9$) waters, however, is congruent and relatively rapid.

There seems to be no correlation between the degree of alteration of nephrite artefacts and their archaeological age or their length of burial. Thus chemical 'weathering', resulting from the action of soil moisture, does not seem to be a viable explanation for the observed alteration. We propose that the alteration consists of congruent dissolution of nephrite, preferentially along grain boundaries, that the alteration takes place in relatively short times, perhaps months, and that it is produced by the action of basic (high pH) rather than acidic solutions. Alteration of select areas of nephrite in sharp contact with unaltered areas may result from differences in the fabric (microstructure) of domains in the polycrystalline material⁵.

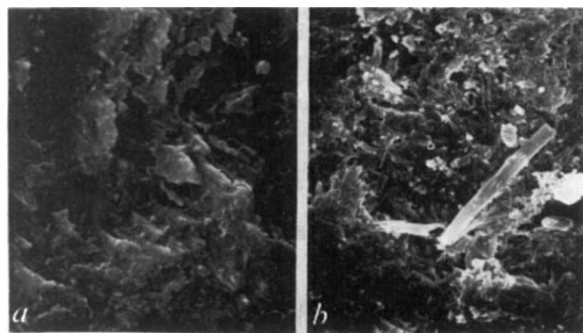


Fig. 3 Scanning electron photomicrographs of *a*, artificially altered nephrite surface (width of field 10 μm); and *b*, altered surface of nephrite artefact (width of field 33 μm).

Conditions suitable for such alteration could be produced and maintained for adequate periods in the tomb environment. Degradation of proteins during liquification of body tissues accompanied by bacterial decay could produce ammoniacal solutions of high pH . In a sealed coffin, such solutions would alter nephrite artefacts buried with the body. The degree of alteration would thus be a function of the degree and duration of contact between the artefact and solution as well as the microfabric of the nephrite. Fabric-marked blades could result from 'wicking' of basic solutions to the blade surface by cloth funerary wrappings.

To test this hypothesis we immersed polished nephrite slabs in solutions containing ammonium hydroxide for several weeks at room temperature. The resulting alteration (Fig. 3a) is essentially identical to, although less severe than, that of the artefact examined (Fig. 3b). The partial alteration of this nephrite, which was totally immersed in the solution, illustrates the importance of the microfabric to the rate of alteration. Domains of randomly oriented tremolite needles are more susceptible to dissolution than those possessing a high degree of orientation. Alteration also occurred more rapidly along fractures and unpolished surfaces, presumably as a result of disruption of oriented microfabrics at fracture surfaces.

The ancient Chinese buried jade with their dead to prevent decomposition of the body—during the Han Dynasty pieces of jade were placed in each of the body orifices to ward off decay. It is ironic that the corpses caused the decay of the jade.

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¹ West, E. H., *Expedition*, Winter 1963, 2–11 (1963).

² Olsen, E. J., *Field Mus. nat. Hist. Bull.*, **42**, 12–13 (1971).

³ Tunni, W., *Chemie Erde*, **12**, 275–303 (1940).

⁴ Krauskopf, K. B., *Introduction to Geochemistry*, 113–116 (McGraw-Hill, New York, 1967).

⁵ Bradt, R. C., Newnham, R. C., and Biggers, J. V., *Am. Miner.*, **58**, 727–732 (1973).

Adsorption of divalent phosphate on hydrous aluminium oxide

The adsorption of phosphate on aluminium and iron hydrous oxides is of practical importance in soils because of its influence on the availability of this nutrient to plants. One aspect of phosphate adsorption is the release of hydroxyl ions into the solution. This paper reports a quantitative relationship between divalent phosphate adsorbed on, and hydroxyl ions released from, hydrous alumina at different phosphate concentrations. From the relationship it is proposed that the phosphate ions are bonded to aluminium atoms forming both monodendate and bridged complexes.

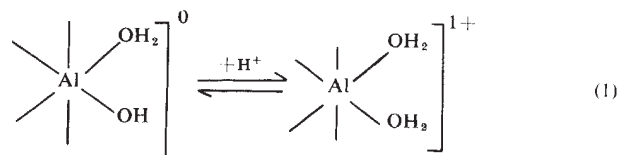
The preparation and properties of the aluminium oxide used have been described elsewhere¹. The sample was predominantly amorphous and had a surface area (B.E.T.) of 197 $m^2 g^{-1}$. The point of zero charge (PZC) pH (ref. 2) was 9.3. The phosphate adsorption and hydroxyl ion release were determined using an automatic titrator³. The adsorption was carried out for 3 h at pH 8.5 in 10 $\mu mol cm^{-3}$ KCl at 30° C under a nitrogen atmosphere. The high pH was chosen so that phosphate in solution existed as divalent ions.

The phosphate adsorption did not reach a maximum (Fig. 1). The theoretical isotherm obtained by using a two-term Langmuir's equation³ was in agreement with the observed adsorption values up to about 280 $\mu mol g^{-1}$, suggesting that at higher values phosphate was adsorbed by different types of sites.

The hydroxyl ions released plotted against phosphate adsorbed (Fig. 2) yielded two straight lines. The ratio of hydroxyl ions released to phosphate adsorbed, R , was 1.44 and 1.07 at less than and greater than 212 μmol of phosphate adsorbed, respectively.

To elucidate the probable mechanism of phosphate adsorption, both the charge characteristic of the oxide solution interface and the phosphate species in solution should be taken into account.

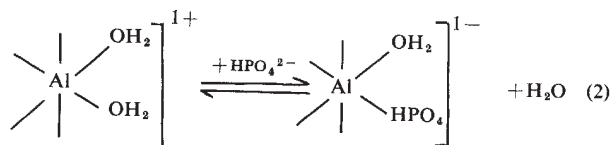
The sign and the magnitude of surface charge on the interface is determined by H^+ and OH^- ions in the absence of other specifically adsorbed anions². At the acid side of the PZC pH the oxide surface can be considered to consist of neutral and positive sites (1).



In the present sample only 40 $\mu mol g^{-1}$ of protons were adsorbed in decreasing the pH from 9.3 (PZC pH) to 8.5.

Considering the great amount of phosphate adsorbed by comparison with the net positive charge, and also the amount of hydroxyl ions released, most of the phosphate was probably adsorbed on neutral sites.

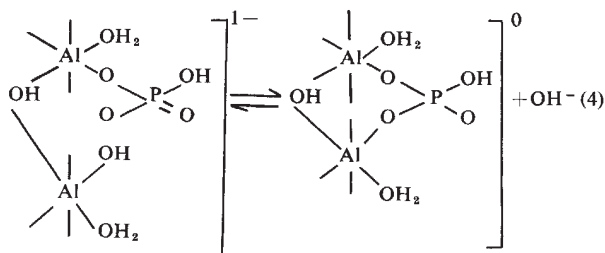
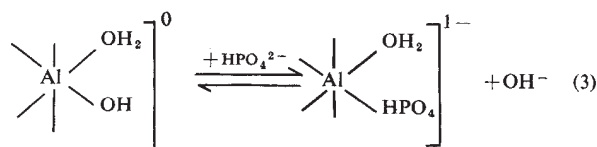
The following ligand exchange reactions can be envisaged for adsorption of phosphate at $< 212 \mu\text{mol}$:



For the reaction as in equation (2) $R = 0$ and for that in equation (3) $R = 1$.

It is probable that the ligand exchange on the neutral adsorption sites is mediated by protons made available by dissociation of the monohydrogen phosphate at the oxide surface⁴. The dissociated form, however, seems to have a transitory existence, for the evidence is that the adsorbed phosphate rapidly acquires a proton from the solvent and equilibrates with the species in solution¹.

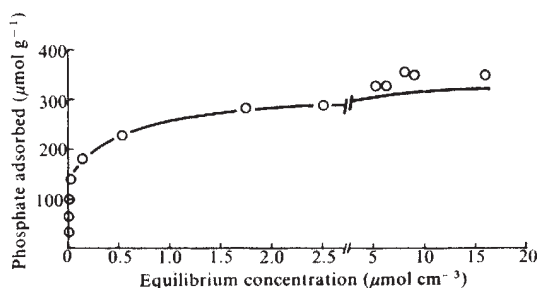
The exchange reactions suggested above do not, however, account for R being > 1 . One possible reaction is the formation of binuclear aluminium phosphate complexes with phosphate bridging across unshared corners of adjacent aluminium atoms (4) following the adsorption as in equation (3).



The value of R for the combined reactions of equations (3) and (4) is 2.

Similar models for the presence of a bridging ligand configuration of the adsorbed phosphate have been proposed from phosphate desorption⁵, kinetics of isotopic exchange⁶ and change in surface charge studies⁷. The R value of 1.44 obtained here is probably the result of reactions (3) and (4) and to a smaller extent reaction (2).

Fig. 1 Phosphate adsorption on hydrous alumina. The curve is the theoretical isotherm obtained by using a two-term Langmuir's equation.



At a $pH \sim 5$, phosphate in solution is mainly present as monovalent ions and is probably adsorbed on hydrous alumina as monodentate ligands¹. With increasing pH the proportion of divalent phosphate in solution, and consequently that adsorbed, will increase resulting in greater amounts of adsorbed phosphate forming ring structure (4). Phosphate held as six membered ring structure can be expected to be more stable than that bonded as monodentate ligands. Hence the lability of the aluminium phosphate complex would decrease with increasing pH . In fact observation to this effect has been made for the isotopic exchange of adsorbed phosphate on goethite⁶. Although the authors explained their results based on acid-base catalysis, disregarding the phosphate species adsorbed, the present results in conjunction with the earlier report¹ indicate that the charge of the phosphate ion also determines the type of bonding of adsorbed phosphate and therefore its lability.

The change in slope of the hydroxyl ion release data observed at 212 μmol of phosphate adsorbed (Fig. 2) suggests a different

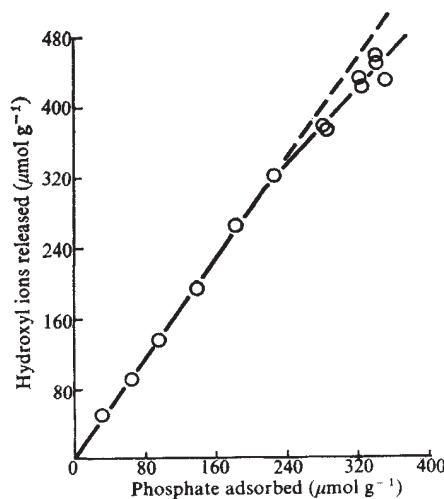
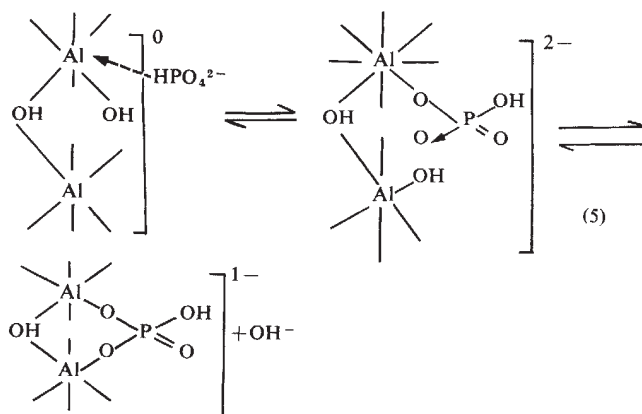


Fig. 2 Hydroxyl ions released plotted against phosphate adsorbed. The two straight lines are drawn down according to the equations:

$$\text{OH}^- = 1.07 (\pm 0.11)P + 79.3 (\pm 33.4) \text{ at } P > 212 \mu\text{mol}$$

mechanism of adsorption at greater values. The fact that the experimental points at high adsorption values deviate from the Langmuir curve corroborates this suggestion.

The change in slope probably indicates disruption of aluminium polymers¹. As the chemical potential of phosphate in solution increases, the phosphate ions will compete with the OH⁻ that are bridging aluminium atoms (ol groups) and eventually displace them forming ring structure (5).



The calculated value of R for such a reaction is 1.0 which agrees with the observed value of 1.07.

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¹ Rajan, S. S. S., Perrott, K. W., and Saunders, W. H. M., *J. Soil Sci.*, (in the press),
² Parks, G. A., and deBruyn, P. L., *J. phys. Chem.*, **66**, 967-973 (1962).

³ Rajan, S. S. S., *N.Z. J. Sci.* (in the press).

⁴ Hingston, A. J., Atkinson, R. J., Posner, A. M., and Quirk, J. P., *Nature*, **215**, 1459-1461 (1967).

⁵ Kafkafi, U., Posner, A. M., and Quirk, J. P., *Soil Sci. Soc. Am. Proc.*, **31**, 348-353 (1967).

⁶ Atkinson, R. J., Posner, A. M., and Quirk, J. P., *J. inorg. nucl. Chem.*, **34**, 2201-2211 (1972).

⁷ Hingston, F. J., Posner, A. M., and Quirk, J. P., *J. Soil Sci.*, **25**, 16-26 (1974).

Reversibility and biological machines

THE application of the traditional, macroscopic arguments of thermodynamics to very small systems (for instance, using individual cross bridges to generate force in striated muscle, which use "one molecule of ATP at a time"¹), has been questioned². It is demonstrated here that they cannot be applied to very small systems without very drastic modification. That is because of the requirements of quantum theory and information theory. The modifications are substantial and inescapable for systems such as individual cross bridges although they become negligible for macroscopic systems.

Consider a machine capable of performing mechanical work. Parallel considerations apply to a device performing electrical work (for example, a fuel cell) but the distinction becomes blurred at the molecular level. For want of a better term I refer to the point of application of the force as the 'piston', although I may be referring to a part of an individual macromolecule undergoing a conformational change. The quantal definition of work, using the Born-Oppenheimer separation of electronic and nuclear motion, has been treated fully elsewhere (B.F.G. in preparation) in a discussion of the quantum mechanics of cross-bridge action.

If the force function is $F(x)$, where x is the position of the piston, then to perform work reversibly, the position of the 'piston' must be measured accurately in order to apply the appropriate external force F_{ex} . Quantum theory and information theory require that a minimum amount of energy is expended in ascertaining position x to within limits $\pm \Delta x$ (where $\Delta x > 0$). The energy cost ΔE is

$$\Delta E \geq hc/4\Delta x \quad (1)$$

according to Brillouin³, where h is Planck's constant and c is the velocity of light. Obviously, ΔE is completely negligible for a macroscopic observation (that is, if Δx is about 1 mm) but for a molecular system ΔE is very important. The uncertainty in the force generated at position x in the process is then

$$\Delta F = F(x) \pm \Delta x(dF/dx) \quad (2)$$

We can assume without loss of generality that $dF/dx < 0$. To perform a reversible extension, we would normally make F_{ex} as close as possible to F (thus ensuring that $F_{ex} = F(x) - |\epsilon|$, with $|\epsilon| \rightarrow 0$ in the limit). Because of the uncertainty the best that becomes attainable is

$$F_{ex} = F(x) + \Delta x(dF/dx) - |\epsilon| \quad (3)$$

The work done by the system is then, in the limit $|\epsilon| \rightarrow 0$

$$W^+ = \int_{x_1}^{x_2} F(x)dx - \Delta x \int_{x_1}^{x_2} \frac{dF}{dx} dx \quad (4)$$

where $F_1 > F_2$ because $x_2 > x_1$. Conversely, in a compression the best that can be attained for the work done on the system is to take $|\epsilon| \rightarrow 0$ in equation (5)

$$F_{ex} = F(x) - \Delta x(dF/dx) + |\epsilon| \quad (5)$$

so that

$$W^- = W_{rev}^- - \Delta x(F_4 - F_3) \quad (6)$$

where $F_4 > F_3$. Clearly, for any cycle involving j steps

$$W = W_{rev} - \Delta x \sum_j |F_j - F_{j+1}| \quad (7)$$

where the correction is always negative. If the net energy input during the cycle is Q , then the conversion factor θ is given by

$$\begin{aligned} \theta &= W/(Q + \Delta E) \\ &= (W_{rev} - \Delta x \sum_j |F_j - F_{j+1}|)/(Q + \Delta E) \end{aligned} \quad (8)$$

By substituting from inequality (1) this gives

$$\theta \leq (W_{rev} - [hc \sum_j |F_j - F_{j+1}|/4\Delta E])/(Q + \Delta E) \quad (9)$$

which can in turn be rearranged in terms of convenient dimensionless quantities α and z

$$\theta/\theta_{rev} \leq (1 - \alpha/z)/(1 + z) \quad (10)$$

where θ_{rev} stands for W_{rev}/Q , that is, the classical conversion factor; the relative or dimensionless energy cost of control

$$z = \Delta E/Q$$

and

$$\alpha = hc \sum_j |F_j - F_{j+1}| / 4QW_{rev}$$

For classical thermodynamics, $\alpha = 0$, that is, Planck's constant is considered negligible, and $z \rightarrow 0$ because the energy cost of achieving reversibility is ignored, whereupon $\theta \rightarrow \theta_{rev}$. For quantal or molecular machines the optimum controlled situation can be calculated by differentiating equation (10) with respect to z (α constant) and setting the derivative equal to zero. The result is

$$z_{max} = \alpha(1 + \sqrt{1 + 1/\alpha}) \quad (11)$$

and this gives the maximum possible value for θ/θ_{rev} as

$$(\theta/\theta_{rev})_{max} = \frac{1 - \{1 + \sqrt{1 + 1/\alpha}\}^{-1}}{1 + \alpha\{1 + \sqrt{1 + 1/\alpha}\}} \quad (12)$$

If more energy than z_{max} is used, then α decreases because of the energy cost of information; if less energy than z_{max} is used θ decreases because of the irreversibility (to dissipation, acceleration of the piston and so on).

For a macroscopic amount of gas, for instance, 1 mol, where $F_j \sim 10^5$ N, and $Q \sim 4 \times 10^4$ J, assuming $\theta_{rev} = 0.9$, $\alpha = 3.5 \times 10^{-28}$ and so, from equation (11), $z_{max} = 1.87 \times 10^{-14}$. (It is important to remember that α and z are dimensionless numbers.) The energy cost of optimal control, Qz_{max} turns out to be

7.5×10^{-10} J, and the resulting conversion factor, θ , is within one part in 10^{14} of the classical value θ_{rev} .

For a cross bridge, or for any comparable molecular machine (because the orders of magnitude are not affected by the detailed mechanics of the system), the situation is very different. Using figures quoted by Huxley⁴ $F_1 \sim 10^{-12}$ N, $Q \sim 6.67 \times 10^{-20}$ J (the 'ATP quantum' of energy/molecule), and again taking $\theta_{rev} = 0.9$, then $\alpha = 12.37$ and $z_{max} = 25.2$. The energy cost of optimal control now turns out to be 1.6×10^{-18} J, which is 25 ATP quanta: $(\theta/\theta_{rev})_{max}$ turns out to be 0.018.

Clearly, any degree of control tending towards reversibility is out of the question for systems as small as this, as in fact they are known to operate at much higher conversion factors than 0.018, both on grounds of theory (B.F.G. unpublished) and of experiment⁵.

Accordingly the conclusion must be reached that the reversible operation of a machine is not in general optimal for the conversion into work of energy entering into the system, except in the limiting case of macroscopic systems where the energy cost of the necessary control becomes negligible compared with the output per cycle of the machine. Further, the optimal conversion process must, in general, occur at a finite rate and, for very small systems for which $z_{max} > 1$, this rate is uncontrolled and determined by the mechanics (quantum or semi-classical) of the system itself.

For intermediate cases the optimal rate will be determined by a compromise between the free running system and its control. Only in the classical limit, $\alpha \rightarrow 0$, $z_{max} \rightarrow 0$, is the optimal process infinitesimally slow. It is interesting to estimate that the minimum size of machine discernible and therefore potentially controllable, by the ATP energy quantum is given by inequality (1) as $2\Delta x = hc/2\Delta E \geq 1.4 \mu m$, which is almost exactly the experimental half-sarcomere length in vertebrate striated muscle, generally regarded as the minimum functional unit under the control of the appropriate Z band.

With regard to the statement of the second law of thermodynamics in a form appropriate to molecular machines, Popper⁶ and McClare⁷ have already stated clearly that the problem involved the necessity of defining work at the microscopic or molecular level. This definition, in purely quantum mechanical terms, is the starting point for Gray and Gonda (B.F.G. in preparation) in a programme of the theoretical investigation of biological molecular machines.

It is necessary to discuss the Born–Oppenheimer separation of nuclear and electronic motion, and the Hellmann–Feynman theorem for time-dependent states. Under certain conditions that are well satisfied in biological machines, it is possible to show that the expectation value of the force as calculated from the Hellmann–Feynman theorem is an adiabatic invariant for non-stationary states involving large nuclear displacements (conformation changes). Work can then be defined simply as a path integral in the usual way. In spite of this, the word 'work' is conspicuously absent from every quantum mechanical textbook I have been able to consult.

From this viewpoint (B.F.G. in preparation) it seems clear that a molecular machine, that is, one which must work below the level of profitable controllability, can operate cyclically and repeatedly in one direction only. If it is regarded as the analogue of a 'heat engine', to use a classical term, it cannot be run backwards as the analogue of a 'heat pump' or 'refrigerator': stretching an actively contracting muscle will not cause cross bridges to traverse their cyclic path in reverse to resynthesise ATP (even assuming the used ADP stayed put) without violating this statement. It is a statement at the molecular level parallel to the macroscopic statement that Joule's experiments on the first law of thermodynamics cannot be carried out in reverse, water will not spontaneously cool and lift a weight.

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- ¹ McClare, C. W. F., *Nature*, **240**, 88 (1972).
- ² Wilkie, D. R., *Nature*, **242**, 606 (1973).
- ³ Brillouin, L., *Sciences and Information Theory* (Academic, New York, 1962).
- ⁴ Huxley, A. F., *Chem. Br.*, **6**, 477 (1970).
- ⁵ Lipman, F., *Adv. Enzymol.*, **1**, 99 (1941).
- ⁶ Popper, K. R., *Br. J. phil. Sci.*, **8**, 151 (1957).
- ⁷ McClare, C. W. F., *J. theor. Biol.*, **30**, 1 (1971).

Time to understand pictures and words

WHEN an object such as a chair is presented visually, or is represented by a line drawing, a spoken word, or a written word, the initial stages in the process leading to understanding are clearly different in each case. There is disagreement, however, about whether those early stages lead to a common abstract representation in memory, the idea of a chair¹⁻⁴, or to two separate representations, one verbal (common to spoken and written words), and the other image-like⁵. The first view claims that words and images are associated with ideas, but the underlying representation of an idea is abstract. According to the second view, the verbal representation alone is directly associated with abstract information about an object (for example, its superordinate category: furniture). Concrete perceptual information (for example, characteristic shape, colour or size) is associated with the imaginal representation. Translation from one representation to the other takes time, on the second view, which accounts for the observation that naming a line drawing takes longer than naming (reading aloud) a written word^{6,7}. Here we confirm that naming a drawing of an object takes much longer than reading its name, but we show that deciding whether the object is in a given category such as 'furniture' takes slightly less time for a drawing than for a word, a result that seems to be inconsistent with the second view.

In each of three conditions with different adult subjects, 96 line drawings of objects or their names written in lower case Letraset (Berling 14 point) were presented one at a time in a tachistoscope, preceded and followed by a mask of haphazard lines and pieces of letters. Each subject saw half the 96 items as words and half as drawings, in alternating blocks of 16 items. Each item was presented as a drawing to half the subjects and as a word to the other half. The subject had never seen the drawing before it was presented. The experimenter said ready or (in the third condition) named a category before each presentation, and after an 800-ms interval the item appeared. A voice key was used to measure response time from the onset of the item.

To discover whether the drawings and words were equally discriminable as visual patterns, in the first condition 16 subjects were shown the items for brief durations, 40, 50, 60, or 70 ms. The durations were presented in a random order, permuted across subjects so that each item was shown equally often at each duration. Subjects named or read the items. The estimated exposure duration required to report 50% of the items correctly was 44 ms for the drawings and 46 ms for the words.

In the remaining two conditions items were presented for 250 ms, at a level well above threshold. Subjects in the second condition ($n=8$) named the object or the word aloud, as rapidly as possible. In the third condition ($n=16$) the experimenter named a category before the item appeared. The subject said yes if the item was a member of the category, as it was on half the trials, and said no otherwise. Altogether there were 18 categories containing two to nine items: for example, food (carrot, pie . . .), clothing (hat, coat . . .), tools (pliers, hammer . . .).

The results of the second and third conditions are shown in Fig. 1. As in earlier reports^{6,7} drawings took longer to

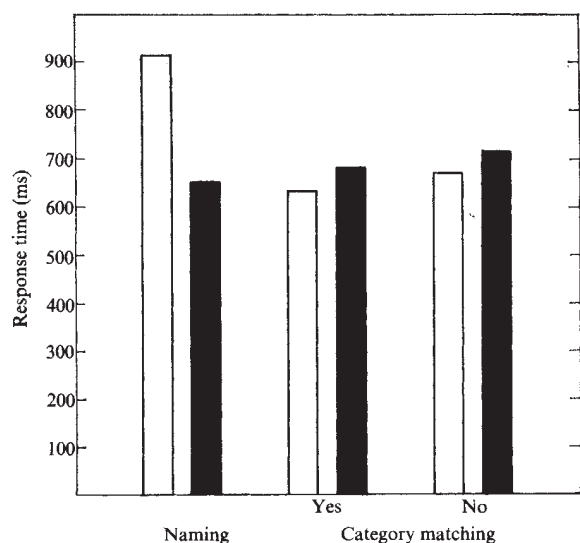


Fig. 1 Mean response time in condition 2 (naming) and condition 3 (matching the item to a spoken category). The white bars are responses to drawings; the black bars, to words. Each bar is based on at least 350 responses; errors and responses that took longer than 2 s (together, less than 5% of the trials) were omitted.

name than words; the mean difference was 260 ms (standard error of the mean difference, 91 ms). The difference was in the same direction for all eight subjects ($P < 0.01$, sign test), and for 93 of the 96 items ($P < 0.001$). In the third condition drawings were categorised faster than words: a difference of 51 ms overall (standard error of the mean difference, 42 ms). The difference was 57 ms for yes responses and 44 ms for no responses. Fourteen out of 16 subjects were faster with drawings than words ($P < 0.01$, sign test). Of the 96 items, 68 were matched faster as drawings ($P < 0.001$).

Recall that the second view of memory asserts that an object has two representations, and that an object's category (a verbal abstraction) is associated with its name and only indirectly with its appearance. If, as that view claims, a drawing must be named implicitly before its category is determined, then in the present experiment one would expect drawings to be categorised more slowly than words^{8,9}, just as they were named 260 ms more slowly. But drawings were not slower than words: they were 50 ms faster. Furthermore, a drawing was categorised much more quickly than it was named, which also makes it unlikely that naming preceded categorising. That finding is, however, not by itself conclusive, since a yes-no matching response may be simpler and so faster than overt naming.

Before one concludes that the second view is untenable, the following four objections must be considered.

(1) Drawings in a given category might have shared certain visual features, so a drawing may have been categorised rapidly on the basis of those features before the subject knew exactly what it was⁹. That is unlikely because the items were chosen to look as diverse as possible, and because at near-threshold durations (first condition) subjects rarely reported a drawing's category but not its name.

(2) Some words (for example, bear, tie, train) were ambiguous, and the first meaning assigned by the subject may not have matched the specified category. An analysis of just the unambiguous items, however, reduced but did not eliminate the advantage of drawings.

(3) Concrete words must be imaged to be categorised—the converse of the naming hypothesis. That seems unlikely, since imaging a word is reported to require at least 0.5 s (ref. 5).

(4) The category of an item is independently associated

to both its name and its appearance. Although our results do not contradict that unparsimonious hypothesis, it would be surprising if a verbal category were more strongly associated with a drawing than a name.

The first view claims that written words and drawings (and presumably also spoken words and objects experienced directly) lead to a common representation in memory, neither word-like nor image-like, and it is that representation which is connected with knowledge of an item's category. In our study, that representation was reached more rapidly from drawings than from words. On this view, naming a drawing is slow because it requires an extra step from the abstract concept to its associated name, whereas naming a word only requires that the word pattern itself be identified¹⁰ and then it may be articulated even before the concept is evoked.

In sum, our results are consistent with the view that knowledge of the category of an object is associated with an abstract idea of the object rather than directly with its name or appearance. Since the name and appearance of an object are also represented in memory, a further question is whether other knowledge one has about an object (such as its typical size or value) is linked to the abstract concept or is directly associated with the name or image. The answer may help to resolve an old question: what are the functions of images and words in thought?

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- Schank, R. C., *Cog. Psychol.*, **3**, 552–631 (1972).
- Anderson, J. R., and Bower, G. H., *Human Associative Memory* (Wiley, New York, 1973).
- Seymour, P. H. K., *Br. J. Psychol.*, **64**, 35–49 (1973).
- Potter, M. C., *Science* (in the press).
- Paivio, A., *Imagery and Verbal Processes* (Holt, Rinehart and Winston, New York 1971).
- Woodworth, R. S., *Experimental Psychology*, (Holt, New York, 1938).
- Fraisse, P., *Percept. mot. Skills*, **11**, 204 (1960); *Amee Psychol.*, **64**, 21–46 (1964).
- Wingfield, A., *Acta. Psychol.*, **26**, 216–226 (1967); *Am. J. Psychol.*, **81**, 226–234 (1968).
- Cohen, G., *Q. J. exp. Psychol.*, **25**, 557–564 (1973).
- Forster, R. I., and Chambers, S. M., *J. verbal Learning verbal Behav.*, **12**, 627–635 (1973).

Sex differences in imagery and reading

STANDARD texts on differential psychology often discuss briefly the existence of sex differences in the ability to perform various cognitive tasks^{1–3}. A rough generalisation is that females perform better than males on verbal tasks (for example, verbal fluency, articulation, spelling), whilst males are superior on visuospatial tasks (for example, maze learning or form-board tasks), although exceptions are plentiful. In view of the emphasis in contemporary cognitive psychology on the pervasiveness of verbal coding in a variety of cognitive tasks, and also current interest in such visuospatial abilities as visual imagery or mental rotation, it seems surprising that no attention has been paid to the possible existence of sex differences.

A possible reason for this neglect is that the sex differences observed in psychometric investigations are usually very small, and sometimes unexpectedly absent, as for example in block design tasks, which would seem to be visuospatial^{4,5}. The inability to obtain clear or even consistent sex differences may well be the result of the impurity of the tasks used. Ostensibly verbal tasks such as syllogistic reasoning or counting can be shown to involve visuospatial imagery^{6,7}; and ostensibly visuospatial tasks such as block design could be successfully performed verbally. Consequently a large underlying sex difference in mode of processing would not necessarily reveal

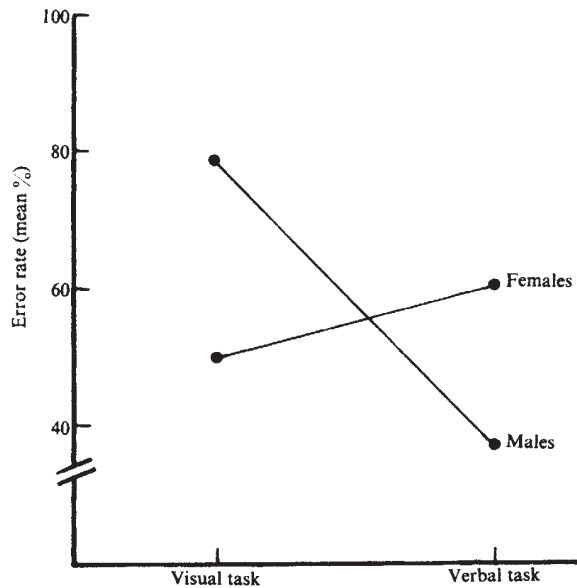


Fig. 1 Percentage of subjects giving the correct response on each task in experiment 1.

itself as a sex difference in level of performance in these tasks. A study of the effect of brain damage on performance on the Kohs block design task⁸ illustrates this possibility. In this study, male performance was more impaired by right-hemisphere than by left-hemisphere damage, whereas for females degree of impairment did not depend on site of damage; furthermore, for females with left-hemisphere damage, but for no other group, there was a significant positive correlation between performance on a verbal task (an aphasia battery) and block design performance. This pattern of results is consistent with extensive verbal involvement in block design performance for females but not for males.

What is needed if these issues are to be properly studied are tasks which are purely verbal or purely visuospatial. We used two such tasks in our first experiment. For the verbal task, subjects were asked to proceed mentally through the alphabet from A to Z, counting the number of letters containing the sound 'ee', including E. No external aids such as speaking or writing were permitted. The subjects were asked to perform as rapidly as possible and the time between the beginning of the task and the utterance of the solution was measured. The visual task, performed under the same conditions, was to proceed mentally through the alphabet from A to Z, counting the number of letters containing a curve in their upper-case form. Since no information about the shape of a letter could assist in deciding whether its name contains the sound 'ee', and since no information about the sounds constituting the name of a letter could assist in deciding whether its printed form contains a curve, we considered that these tasks were to a sufficient degree purely verbal and purely visual. The subjects were 75 right-handed undergraduates at the University of Reading, 38 females and 37 males; they were tested individually with both tasks.

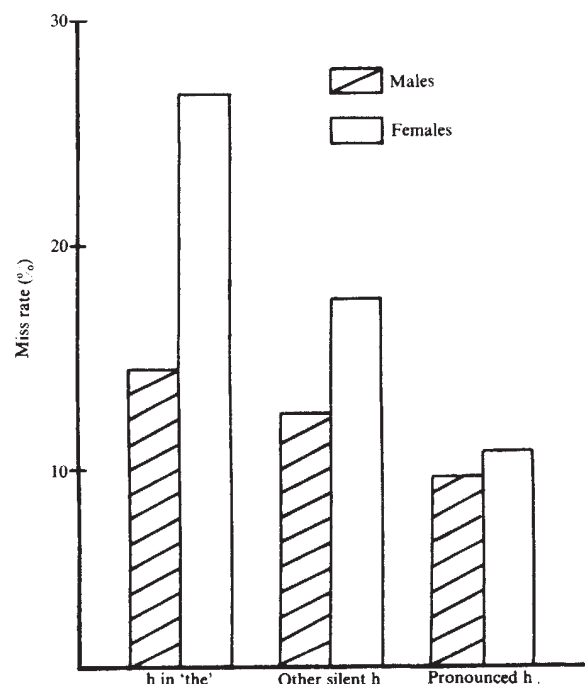
Females completed the verbal task more rapidly than males (22.9 s compared with 23.7 s) whilst males completed the visual task more rapidly than females (21.5 s compared with 22.4 s), but this interaction was not statistically significant, because of the very large within-sex individual differences in completion times. A significant effect was, however, obtained in error rates. Surprisingly, many subjects gave incorrect responses, no doubt because they had been asked to perform the task as quickly as possible and were being timed. The percentage of subjects responding correctly to each task is shown in Fig. 1. Significantly more females than males were correct on the verbal tasks ($\chi^2=3.85$, $P < 0.05$). Significantly more males than females

were correct on the visual task ($\chi^2=6.50$, $P < 0.05$). The sex difference in error rates is quite large, for both tasks.

To determine whether such marked differences are confined to tasks involving imagery, we next used a task developed by Corcoran⁹. He found that subjects asked to scan through a passage of prose crossing out all occurrences of the letter 'e' overlooked unpronounced e's (as in 'late') much more often than pronounced e's (as in 'let'). This indicates that both verbal and visual analysis is occurring in this apparently visual task. If females rely strongly on verbal analysis, and males on visual analysis, then females should have more difficulty than males in detecting unpronounced letters, since verbal analysis cannot detect such letters. We used 10 male and 10 female undergraduates and asked each to scan through six photographically enlarged pages from novels, crossing out all occurrences of the letter 'h'. They were given 2 min for each page, with 2 min rest between pages. The results are shown in Fig. 2. For pronounced targets, there was a negligible difference between male and female miss rates; for unpronounced targets, female miss rates were much higher than male miss rates. This interaction between target type and sex was significant ($F = 4.15$, $P < 0.05$) as was the main effect of target type ($F = 6.97$, $P < 0.01$) and sex ($F = 25.50$, $P < 0.01$).

This result implies that phonological coding during reading is more prevalent in women than in men, and this was investigated more directly in our third experiment. If we use the term 'internal lexicon' to refer to the stored body of knowledge concerning the words of the language, and the term 'lexical entry' to refer to a subset of the information referring to a particular word (information about how the word is spelled, how it is pronounced, what it means and so on), then the task in visual word recognition is to extract information from a visually presented sequence of letters, and to use this information to locate the lexical entry belonging to the word being viewed (for example by searching the lexicon for an entry containing this information). Given this theoretical framework, we can ask what the nature of the information is. An enduring conflict in the study of reading has been between those who consider that the information is visual, and those who consider that it is phonological, that is, that a letter sequence is converted to a phoneme sequence (using a system of grapheme-phoneme

Fig. 2 Miss rates in experiment 2.



correspondence rules) before lexical access. There is evidence in favour of both views; this conflicting evidence can be reconciled by proposing that both forms of access are used, simultaneously and independently, and that whichever system locates the required lexical entry first is responsible for word recognition on that particular occasion. Given this, we might expect a sex difference in the relative efficacy of the two systems; word recognition mediated by phonological encoding might be the predominant method of lexical access for women, and visually-mediated word recognition might be the predominant method for men.

We investigated this by using a lexical-decision task¹⁰. On each trial in this experiment, the subject was presented with a row of letters and asked to press a YES key as quickly as possible if the stimulus were an English word, otherwise to press a NO key. The stimuli were presented on an oscilloscope screen controlled by a PDP-12 computer, which also measured the time between display onset and response, recorded which response was made, and printed a data analysis at the end of each subject's session. The subjects were 10 male and 10 female undergraduates and research students at the University of Reading.

A total of 156 letter sequences was presented, in a different random sequence to each subject. Of these, 39 were homophonic words, that is, for each of them there existed another English word with the same pronunciation and a different spelling (for example, SUITE, PAWS or URN); 39 were non-homophonic English words, each matched with one of the homophones on number of letters, number of syllables, word frequency and part of speech; 39 were homophonic non-words constructed so that for each of these non-words there existed two English words with the same pronunciation but different spellings (for example, HORL, LAKS, or THROO); and 39 were non-homophonic non-words, each derived from one of the homophonic non-words by changing one letter so that the resulting letter sequence was still perfectly pronounceable but was not pronounced like any English word.

If access to the internal lexicon is ever phonological, it will be more difficult to say NO to HORL than to DORL, since the phonological system will incorrectly locate a lexical entry in the case of HORL but not in the case of DORL¹⁰. Furthermore, if phonological access is more prevalent in women than in men, then the difficulties engendered by homophonic non-words such as HORL should be more severe for women than for men.

Mean latencies for correct NO responses were 578 ms for men and 737 ms for women when homophonic non-words were used, and 545 ms for men and 647 ms for women when non-homophonic non-words were used.

The NO response was thus slower for non-words which sounded like English than for those which did not. This was true for every one of the twenty subjects. The effect was larger for women than for men (33 ms compared with 90 ms), and this difference was statistically significant (Mann-Whitney $U = 23$, $P < 0.05$). In the case of words, response latency was not influenced by whether or not a word was a homophone; the only significant effect was that women were about 100 ms slower than men in responding YES to words.

The results with non-words indicate that phonological access to the lexicon is used to some extent by both sexes, but more by women than by men. If phonological access is completed before visual access more often in women than in men (for example, because women are more efficient or faster than men at applying phonological rules to letter sequences), the sound of the word will have more effect on women than on men; as was the case.

Whether these sex differences are present early in life remains to be determined, but if the same pattern of sex differences appears in young children, this finding may have educational implications. For example, it may prove efficient to teach girls to read by a phonological method ('phonics') and boys to read by a visual method (for example, the 'whole-word'

method). Some of the sex differences in cognition discussed earlier may in fact be present very early in life^{2,11}. Furthermore male superiority in spatial tasks is evident in rats as well as men¹¹, and seems to be susceptible to hormonal influences in rats and in men^{11,12}. If relatively pure tests of visual and phonological processing, such as those used in experiment 1, can be devised which are suitable for use with children before they learn to read, it may be possible to obtain satisfactory evidence concerning the origins of the sex differences in cognition described in this paper.

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Received November 20, 1974.

- ¹ Anastasi, A. *Differential Psychology* (3rd ed.) (Macmillan, Basingstoke, 1958).
- ² Maccoby, E. (ed.) *The Development of Sex Differences* (Tavistock, 1967).
- ³ Terman, L. M., and Tyler, L. E., *Manual of Child Psychology* (edit. by Carmichael, L.) (2nd ed.) (Wiley, New York and London, 1954).
- ⁴ Gainer, W. L., *Calif. J. educ. Res.* 13, 9, (1962).
- ⁵ Norman, R. D., *J. Consult. Psych.* 17, 411 (1953).
- ⁶ De Soto, C., London, M., and Handel, S., *J. Pers. Soc. Psych.* 2, 513 (1965).
- ⁷ Monty, R. A., Taub, H. A., and Laughery, K. R., *J. exp. Psych.* 69, 224 (1965).
- ⁸ McGlone, J., and Kertesz, A., *Cortex* 9, 313 (1973).
- ⁹ Corcoran, D. W. J., *Nature* 210, 658 (1966).
- ¹⁰ Rubenstein, H., Lewis, S. S., and Rubenstein, N. A., *J. verb. Learn. verb. Behav.* 10, 645 (1971).
- ¹¹ Buffery, A. W. H., and Gray, J. A., in *Gender Differences* (edit. by Ounsted, C., and Taylor, D. C.) (Churchill, 1972).
- ¹² Dawson, J. L. M., *Internat. J. Psych.* 2, 115 and 171 (1967).

A model illustrating the importance of timing in the regulation of breathing

THE effectiveness of afferent volleys from arterial chemoreceptors in stimulating inspiration depends on the precise timing of their arrival within the central nervous system^{1,2,16}. Torrance¹ suggested that variation in the degree of mismatching of the actual with the optimum timing of volleys would provide an extra element of fine control in the respiratory system. I have developed a scheme which is based on this suggestion and also on the following experimental observations.

(1) In the cat the afferent impulses from chemoreceptors are grouped in bursts the peaks of which correspond in time to the middle of the rising phase of the oscillations of carotid arterial P_{CO_2} (refs 3 and 4); peak afferent discharge should thus be related to mid-expiration, the two events being separated in time by the lung-to-carotid body circulation time, itself relatively constant under a single set of circumstances, and in normal men commonly occupying the duration of nearly two respiratory cycles⁵.

(2) A burst of chemoreceptor discharge in the cat is only effective in augmenting inspiration if it falls within a certain part of the cycle, the period of central inspiratory excitability (PCIE), which is closely related to inspiration itself. Inspiration cannot be excited by this pathway during the rest of the cycle, the system behaving as if memory is short^{1,2}. Afferent volleys arriving outside the PCIE do, however, prolong expiration^{1,16}.

(3) In man, the respiratory system is capable of detecting differences in the alveolar P_{CO_2} time profile so brief as to occupy only one-third of the whole cycle. This property is almost certainly mediated by arterial chemoreceptors⁶.

(4) Also in man, with changes in chemical drive from any source, tidal volume (V_T) and the duration of expiration (T_E), and hence total cycle duration (T_T) are inversely related⁷. The inverse relation holds good on a breath-by-breath basis⁸.

A crude model has been constructed (Fig. 1) using squared paper and following some arbitrary quantitative rules: (a) initially steady eupnoeic inspiration and expiration occupy 1 and 2 s respectively; (b) PCIE coincides exactly

with inspiration; (c) the afferent volleys also last for 1 s; (d) a volley stimulates inspiratory volume and shortens expiratory duration in proportion to the overlap between it and PCIE; and (e) with no overlap the volley is ineffective, inspiratory volume is decreased and expiratory duration lengthened from the eupnoeic values. In these circumstances, the residual ventilation is prevented from falling below 70% of the eupnoeic level by the slowly-acting central drive from (CO_2 , H^+).

In cases 1 and 2 (Fig. 1) the lung-to-carotid body circulation time is such that, with the initial steady breathing, the burst of afferent impulses follows the PCIE of the next-but-one respiratory cycle and overlaps it by one-fifth. In cases 3 and 4 the circulation time is lengthened so that the afferent burst is delayed sufficiently to overlap the PCIE of the next-but-two eupnoeic cycle; the overlap, however, although again one-fifth, is reversed and the burst precedes the PCIE.

After four steady eupnoeic cycles (Fig. 1) an extrinsic irregularity, comprising a long (cases 1 and 3) or short (cases 2 and 4) breath, disturbs the rhythm; such irregularities occur continually in normal human breathing⁹⁻¹¹. The regular time-base provided by the spacing of the afferent bursts is not disturbed until two cycles later, but the overlap between the bursts and the PCIE is altered after only one cycle; the consequences for V_T and T_E depend on the circumstances (Fig. 1).

In cases 1 and 2, after the disturbance, a large breath of short duration alternates with one or two small breaths of long duration but the alternation of the overlap between afferent bursts and PCIE is such that correction by the timing mechanism is towards the mean, and regularity (case 2), or regular non-uniformity (case 1), of the time base is quickly re-established. Over two or three cycles in cases 1 and 2, respectively, the mean quotient $V_T/(T_I + T_E)$, (=mean ventilation, $\bar{V}$) remains at the predisturbance value. In other words, negative feedback results and overall stability of ventilation is achieved.

On the other hand in cases 3 and 4 (afferent bursts preceding PCIE), the initial steady eupnoea turns out to be unstable. After an extrinsic long breath (case 3), PCIE falls so far behind the afferent burst as to lose touch completely and the mean ventilation stabilises at the minimum value of 70% of eupnoea; the time-base is slowed and is incapable of reversing the situation. After an extrinsic short breath

(case 4), the burst-PCIE overlap is increased and the next breath is also shortened. A complex oscillation of response ensues, with the pattern repeating every six breaths; the time-base is irregular, mean ventilation settles 33% above the eupnoeic value and the time relation between burst and PCIE may reverse. Although the details of the pattern depend on the numerical values assumed, the general features are found with most of the likely combinations. Cases 3 and 4 exhibit an element of positive feedback, limits being set by the maximum and minimum possible effectiveness of the chemoreceptor drive under the prevailing conditions of blood-gas tensions.

Models of this type are consistent with a number of observable phenomena of normal human breathing. The enhancement of hypoxia sensitivity in even mild exercise¹² in the absence of any increase in the average impulse frequency in the chemoreceptor afferents¹³, could be due to 'sharpening' of the grouping of impulses into bursts. Stability of ventilation would, however, require that the PCIE precede the bursts, as in cases 1 and 2.

At low ventilations, T_E varies much more with small changes in V_T than when ventilation is high⁷. The model would thus predict the well-known irregularity of breathing in the short term during eupnoea, especially when inhalation hypoxia is present. It also predicts the greater regularity when ventilation is stimulated by CO_2 inhalation, although other factors are certainly involved here.

In the periodic breathing of high altitude the period of the swing is about 0.75–1 min, a time more appropriate to the slow, well-damped negative-feedback loop involving the intracranial (CO_2 , H^+) receptor system, than to the fast, poorly-damped loop involving peripheral chemoreceptors. The latter, however, is an essential component as its inactivation by breathing oxygen abolishes the periodic breathing. A scheme like that of cases 3 and 4 would resolve the paradox: with the afferent bursts preceding PCIE, a slight disturbance would promote the maximum or minimum situation, with ventilation and metabolism mismatched. Correction of the mismatching by the central (CO_2 , H^+) loop would have a long time constant but the correction itself would activate the peripheral loop which, by positive feedback, would quickly flip the whole system over into the other extreme state. Periodic breathing is most marked in recumbency, especially in sleep, during which lung-carotid circulation time is slightly lengthened and there are few

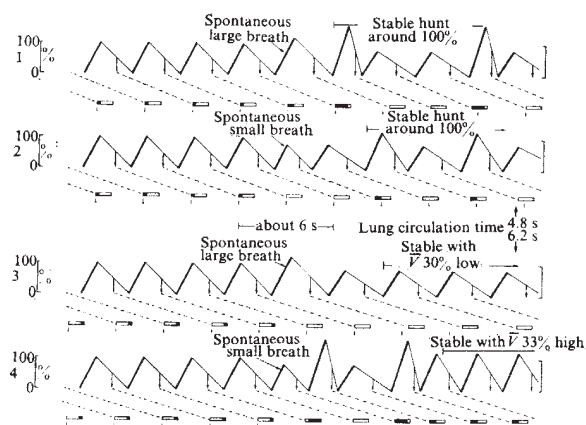


Fig. 1 The timing model: simulated time relation between respiratory movements, scaled to percentage of control, and chemoreceptor bursts of activity. Inspiration upwards (bold lines) with four steady eupnoeic cycles at left of each line. Afferent volleys (rectangles) last 1 s and each is connected by dashed line to the middle of the expiration (downward arrow) that initiated it. Volleys only stimulate inspiratory volume and flow; and shorten expiratory duration when they overlap PCIE, which coincides with duration of inspiration. The degree of overlap is indicated by blackening of the volley symbols: + $\Delta 0.2$ s of overlap results in a + $\Delta 20\%$ tidal volume and - $\Delta 20\%$ expiratory duration. Inspiratory duration (1 s) is unaffected. With no overlap, chemoreceptor bursts are ineffective and central respiratory drive prevents ventilation from falling below 70% of eupnoea. Cases 1 and 2: circulation time is 4.8 s, that is, slightly less than the duration of two respiratory cycles; thus volley overlaps PCIE from behind. An extrinsic long or short breath initiates changes in overlap which do not change mean ventilation, $\bar{V}$, although in the absence of damping in the model the short term hunt would continue indefinitely. Cases 3 and 4: circulation time is 6.2 s, that is, slightly longer than the time for two respiratory cycles; thus volley overlaps PCIE from in front. An extrinsic long or short breath is followed by changes that reveal the intrinsic instability. In case 3, the time-base provided by the volleys is lengthened and mean ventilation falls. In case 4, the volley-PCIE overlaps vary widely, breathing is irregular and mean ventilation high. These states continue until corrected by slow changes in the central drive; over-correction and longer term hunting are likely to occur.

irregularities to disturb the hunting pattern, although other factors are probably involved. It is also possible that some pathological states result from disorders of the mechanism described.

The model is clearly over simplified: thus PCIE and the afferent bursts have been given duration but not intensity. The time profile of the PCIE might resemble that of the phrenic neurogram and the profile of the bursts is probably like a skewed sine wave⁴. The bursts could also vary in amplitude, and rhythmic or periodic cardio-respiratory events could affect the transit times of the blood-gas oscillations from lungs to receptors. Further developments will require more sophisticated computation.

An important feature of the mechanisms whereby phasic afferent activity could produce the effects described is that there should be little or no central memory of earlier bursts; this could be achieved if the connections were very simple. Indeed, Davies and Edwards¹⁴ found few synapses between the incoming fibres and the central inspiratory neurones. The nature of the effect on T_E is not known: Clark and Euler¹⁵ report that induced changes in V_T lead to changes in T_I , which they regard as being linked to T_E . In man, however, T_E may respond to changes in chemical drive while T_I remains constant⁷.

The relative contribution of the mechanism to short term regulation in normal man at rest at sea level is probably small and possibly even trivial^{8,12}. During exercise, however, 'sharpened' bursts of afferent activity could provide a back-up device such that if ventilation, and particularly its frequency component, were to fall momentarily below the requirements of metabolic exchange, both would receive an extra 'push from behind' so that the proper separation between afferent burst and PCIE would rapidly be re-established.

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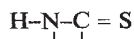
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Received November 6, 1974.

- ¹ Black, A. M. S., and Torrance, R. W., *Resp. Physiol.*, **13**, 221-237 (1971).
- ² Band, D. M., Cameron, I. R., and Semple, S. J. G. *J. Physiol., Lond.*, **211**, 479-494 (1970).
- ³ Band, D. M., Saunders, K. B., and Wolff, C. B., *J. Physiol., Lond.*, **218**, 73-74P (1971).
- ⁴ Willshaw, P., *Abstr., Satellite Symposium on Arterial Chemoreceptors* (Srinagar, Kashmir, 1974).
- ⁵ Cunningham, D. J. C., and Drysdale, D. B., *ibid.*
- ⁶ Cunningham, D. J. C., Howson, M. G., and Pearson, S. B., *J. Physiol., Lond.*, **234**, 1-28 (1973).
- ⁷ Cunningham, D. J. C., and Gardner, W. N., *J. Physiol., Lond.*, **227**, 50-51P (1972).
- ⁸ Gardner, W. N., *J. Physiol., Lond.*, **242**, 75P (1974).
- ⁹ Prihan, I. P., *J. Physiol., Lond.*, **166**, 425-434 (1963).
- ¹⁰ Cunningham, D. J. C., Pearson, S. B., and Gardner, W. N., *Arch. Fisiol.*, **69**, suppl., 433-446 (1973).
- ¹¹ Newsom-Davis, J., and Stagg, D. T., *Arch. Fisiol.*, **69**, suppl., 418-423 (1973).
- ¹² Cunningham, D. J. C., *Q. Rev. Biophys.*, **6**, 433-483 (1974).
- ¹³ Davies, R. O., and Lahiri, S., *Resp. Physiol.*, **18**, 92-100 (1973).
- ¹⁴ Davies, R. O., and Edwards, M. W., *Abstr., Satellite Symposium on Arterial Chemoreceptors* (Srinagar, Kashmir, 1974).
- ¹⁵ Clark, F. J., and Euler, C., *J. Physiol., Lond.*, **222**, 267-295 (1972).
- ¹⁶ Eldridge, F. L., *J. Physiol., Lond.*, **222**, 319-333 (1972).

PTC taste blindness and the taste of caffeine

TASTE thresholds for the bitter substance PTC (phenylthiourea or phenylthiocarbamide) and related compounds containing the grouping



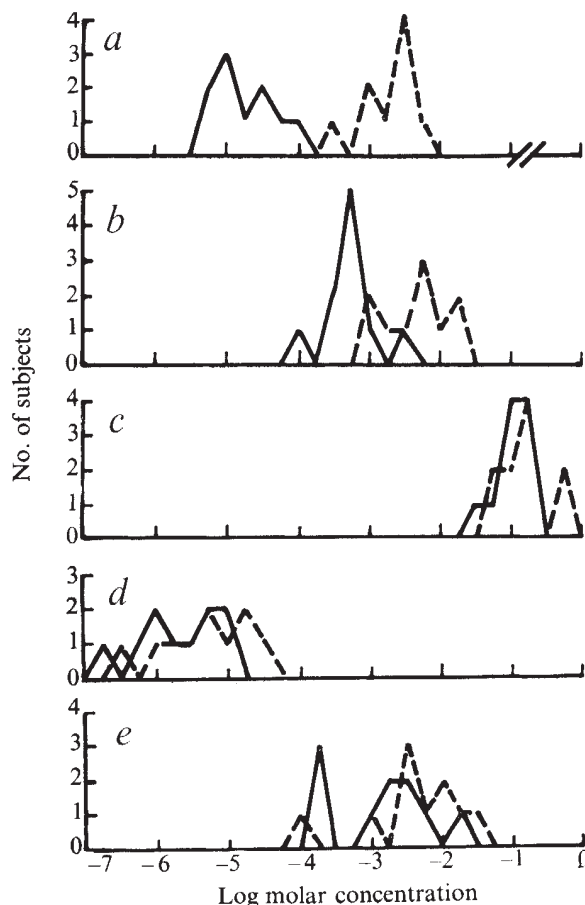
(HNCS) show a bimodal distribution, leading to the designation 'tasters' for the more sensitive individuals and 'non-tasters' or 'taste blind' for the less sensitive. Genetic and population studies have generally attributed this to a simple Mendelian dominance system in humans, non-human primates and some rodent species. All other bitter and non-bitter compounds tested so far have produced Gaussian distributions of thresholds^{1,2}. We have found now, however, that sensitivity to the taste of PTC predicts sensitivity to caffeine, a common bitter substance that lacks the HNCS grouping. This was shown by

threshold measurements and magnitude estimation of supra-threshold concentrations.

We tested ten non-tasters (the first ten undergraduates uncovered by a rough PTC threshold screening procedure) and ten tasters. Each subject's threshold was measured for each of five compounds: PTC, quinine hydrochloride (QHCl), urea, caffeine and sodium chloride (NaCl). The method was a modified Harris-Kalmus procedure³ in which the subject was given eight cups; four contained water (Hydro Service ultrapure water with resistance greater than 18 MΩ cm⁻³) and four contained a particular concentration of the substance under evaluation. The subject rinsed his mouth thoroughly with water before sampling from each of the eight cups which he sorted into two groups: those with a taste and those without. The concentration was decreased successively by 0.25 log steps until the subject failed to sort correctly.

Suprathreshold concentrations were flowed on to the extended tongue at a rate of 4 ml s⁻¹ through a McBurney gravity flow system⁴. The stimuli were always preceded by a 20 s water rinse and were presented, twice each, in random order. Subjects were asked to assign numbers proportional to the perceived intensities of the stimuli⁵. For example, if the first stimulus was judged '10 bitter' and the second stimulus seemed twice as intense, then it was to be judged '20 bitter'. Solutions in both phases of the experiment were warmed to 34° C. Since NaCl and PTC sensitivity are not related, judgments of the bitter substances were expressed relative to NaCl in the following way. The geometric mean of the magnitude estimates for the four NaCl concentrations was obtained for each subject. This mean for each subject was made equal to ten by multiplying with an appropriate factor. The subject's magnitude estimates of the bitter substances were then multiplied

Fig. 1 Harris-Kalmus thresholds for PTC (a), caffeine (b), urea (c), QHCl (d), and NaCl (e) for tasters and non-tasters of PTC. *One subject was unable to taste even 0.018 M PTC, the highest concentration used. —, Tasters; ---, non-tasters.



by the same factor. This transformation had no effect on the ratios among magnitude estimates. It merely decreased variability contributed by the absolute size of numbers chosen by the subjects.

Figure 1 shows the thresholds for tasters and non-tasters of PTC. The bimodal distribution for PTC with peaks at 0.00001 M and 0.003 M PTC is typical. The bimodal distribution that resulted for caffeine, however, has not been reported before. Even more interesting, the caffeine thresholds were correlated with the PTC thresholds (Spearman rank correlation coefficient = 0.83, $P < 0.001$). None of the other substances produced a clear bimodal threshold distribution nor were the thresholds correlated with those for PTC.

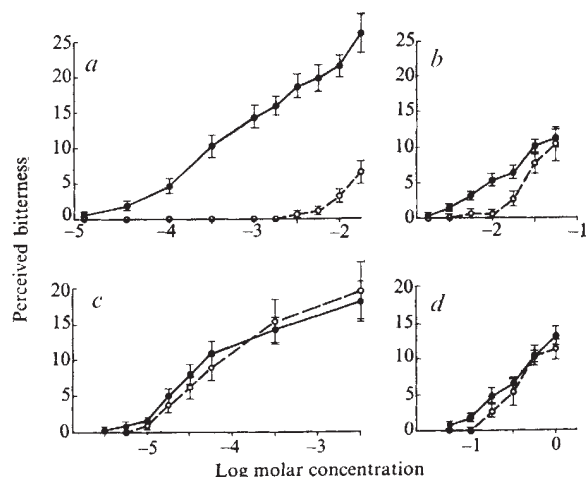


Fig. 2 Perceived bitterness of PTC (a), caffeine (b), QHCl (c), and urea (d) for tasters and non-tasters of PTC. ●, Tasters; ○, non-tasters.

Figure 2 shows the perceived bitterness of suprathreshold concentrations of PTC, caffeine, urea and QHCl for tasters and non-tasters of PTC. The functions for PTC show the large difference in sensitivity between tasters and non-tasters to be expected from the PTC threshold results. The functions for caffeine also confirm the lesser difference between tasters and non-tasters shown by the caffeine threshold results. The difference is, however, restricted to the lower concentrations (t tests, $P < 0.01$ for 0.056 to 0.03 M caffeine). The steepened slope of the non-taster caffeine function is reminiscent of the recruitment of loudness in typical nerve deafness⁶. At sufficiently high concentrations, the two PTC functions might meet as the caffeine functions do; however, this possibility cannot be tested because of the limited solubility of PTC.

The lower concentrations of urea were also perceived as slightly less bitter by the non-tasters (t tests, $P < 0.01$ for 0.1 and 0.18 M urea). This suggests that the taste of urea, like that of caffeine, may be related to the taste of PTC even though the peaks of the urea threshold distributions for tasters and non-tasters cannot be resolved. Neither caffeine nor urea contains the HNCS grouping. There are many bitter substances that have not been examined for possible connection with PTC sensitivity, so it now seems likely that there must be others that will also taste less intense to PTC non-tasters.

Beidler and Gross⁷ have argued that current knowledge about the nature of taste receptor sites leads to the conclusion that "there exists no single receptor site peculiar for all molecules representative of any particular taste quality". Our psychophysical results add evidence for the existence of at least two receptor sites underlying the taste of bitter, with the 'PTC site' largely absent in non-tasters and the 'quinine site' present in

both tasters and non-tasters of PTC. Caffeine and possibly urea seem to stimulate PTC sites. This interpretation is also supported by cross-adaptation results reported by McBurney and colleagues. Adaptation to QHCl does not substantially reduce the bitterness of PTC (ref. 8) although among sweet, sour and salty substances, cross-adaptation is virtually complete^{9,10}. That is, adaptation to a substance of one of those qualities reduces the taste intensity of other substances of the same quality.

The possibility of multiple receptor sites underlying a single taste quality poses an interesting problem in sensory coding. Why should PTC and QHCl both taste bitter if they are coded by different receptor sites? The cross-fibre patterning hypothesis^{11,12} gives one answer. Both substances may stimulate largely the same population of nerve fibres even if the receptor sites by which they accomplish this stimulation are different. That is, quality is determined by the pattern of neural activity received by the central nervous system.

A series of earlier studies seemed to support the conclusion that a taster needed his own saliva to taste PTC¹³⁻¹⁶. In the classic study, Cohen and Ogden¹⁵ dried the tongues of tasters with air from an atomiser. Although this procedure rendered their tasters insensitive to PTC, it also rendered half of their control subjects insensitive to saturated solutions of saccharin and salt. They concluded that "... saliva aids in many taste sensations ..." but they could have concluded instead that air drying the tongue interferes with normal taste perception. In all phases of our experiment, PTC was tasted after a thorough water rinse. Our threshold values are similar to those obtained without a rinse condition^{1,16}. We conclude that saliva is not crucial to the ability to taste PTC.

One implication of our results concerns the bitter taste of caffeine in coffee. Coffee tastes bitter because it contains several bitter constituents. The concentration of the caffeine in an average cup of brewed coffee, 0.003-0.004 M caffeine¹⁷, is below or near the threshold of most non-tasters but above the threshold of most tasters. Caffeine cannot, therefore, add a significant amount of bitterness to the already bitter taste of coffee for the non-taster of PTC. It may or may not add some bitterness for tasters depending on the intensities of the other constituents. Fisher and Griffin¹⁶ have shown that taste sensitivity to some bitter compounds is correlated with systemic pharmacological sensitivity. It would be particularly interesting to see if taste sensitivity to caffeine predicts sensitivity to the pharmacological effects of caffeine as a stimulant.

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- 1 Fischer, R., in *The Chemical Senses and Nutrition* (edit. by Kare, M. R., and Maller, O.), 61-81 (Johns Hopkins University Press, Baltimore, 1967).
- 2 Klein, T. W., and De Fries, J. C., *Nature*, **225**, 555 (1970).
- 3 Harris, H., and Kalmus, H., *Ann. Eugen.*, **15**, 24-31 (1949).
- 4 McBurney, D. H., and Pfaffmann, C., *J. exp. Psychol.*, **65**, 523-529 (1963).
- 5 Stevens, S. S., *Percept. Psychophys.*, **6**, 302-308 (1969).
- 6 Stevens, S. S., *J. acoust. Soc. Am.*, **39**, 725-735 (1966).
- 7 Beidler, L. M., and Gross, G. W. in *Contributions to Sensory Physiology* (edit. by Neff, W. D.), 5, 97-127 (Academic, New York, 1971).
- 8 McBurney, D. H., Smith, D. V., and Shick, T. R., *Percept. Psychophys.*, **11**, 228-232 (1972).
- 9 Smith, D. V., and McBurney, D. H., *J. exp. Psychol.*, **80**, 101-105 (1969).
- 10 McBurney, D. H., *Percept. Psychophys.*, **11**, 225-227 (1972).
- 11 Pfaffmann, C., *J. Neurophysiol.*, **18**, 429-440 (1955).
- 12 Erickson, R. P. in *Olfaction and Taste* (edit. by Zotterman, Y.), 1, 205-213 (Pergamon, New York, 1963).
- 13 Fox, A. L., *Proc. natn. Acad. Sci. U.S.A.*, **18**, 115-120 (1932).
- 14 Salmon, T. N., and Blakeslee, A. F., *Proc. natn. Acad. Sci. U.S.A.*, **21**, 78-90 (1935).
- 15 Cohen, J., and Ogden, D. P., *Science*, **110**, 532-533 (1949).
- 16 Fischer, R., and Griffin, F., *Drug. Res.*, **14**, 673-686 (1964).
- 17 Ritchie, J. M., in *The Pharmacological Basis of Therapeutics* (edit. by Goodman, L. S., and Gilman, A.), 358-370 (Macmillan, New York, 1970).

Processing of positional information in the human visual system

CONTOURS seem to be shifted away from the edge of a previously seen figure. Ganz¹ considered this apparent displacement to be caused by lateral inhibition between the contours of an after image of the inducing figure and the test figure itself.

Similarly, two lines of different orientation that are presented simultaneously seem to be displaced from one another in orientation. To account for the perceptual expansion of acute angles Blakemore, Carpenter and Georgeson² postulated a superposition of excitatory and inhibitory distributions of activity among the population of orientation detectors in the visual cortex. Superposition would cause the peaks of the distributions to be shifted slightly apart and hence cause the brain to deduce that the angle is larger than it is.

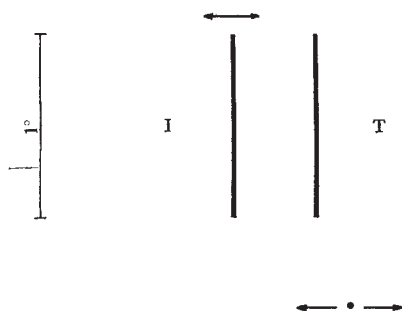
Two parallel lines at threshold facilitate each other when they are separated by less than say 2.5', whereas inhibition occurs for larger separations up to about 10' (ref. 3). A line weighting function may be computed from the change of increment threshold for one line as a function of separation from the additional line. The shape of this function compares fairly well with other estimates of the spatial extent of excitatory and inhibitory interactions in the light-adapted fovea.

Line weighting functions may be tentatively ascribed to the combined spread of excitation and inhibition among line detectors. Kulikowski and King-Smith⁴ presented evidence that there exist line detectors which are distinguishable from grating detectors: the 'grating sensitivities' of the two detectors as revealed by subthreshold summation are different. This result receives support from the observation that frequency selective preadaptation of line detecting mechanisms shows no interocular transfer as it is found following adaptation of grating detecting mechanisms⁵. Repulsion between parallel lines separated by less than 10' would then be expected by analogy with the model of Blakemore *et al.* We performed an experiment to test this prediction.

The stimuli were two parallel, vertical lines, which were 1° long and 1' wide at a viewing distance of 114 cm, presented on a 6° square background, which had a luminance of 36 cd m⁻² (Fig. 1). The position of the inducing line (I) was set by the experimenter, after which the observer adjusted the horizontal position of a pair of 1' diameter lights, which were 0.5° above and below the test line (T), until they appeared to be aligned with T.

The observer, whose position was stabilised by a chin rest, was allowed to inspect freely the test field during his observations. Starting alternately from different directions, it usually took the subject seconds to set the dots. He gave two readings

Fig. 1 Stimulus configuration: the separation between the test line T and the inducing line I was set by the experimenter. The subject had to assess the position of T by aligning the dots and the line.



for each position of a series of increasing or decreasing line separations. The procedure was repeated in reverse order. Thus four values were obtained for each position of I in one experimental session.

Contrary to expectation, no displacement effects were obtained when test and inducing lines were of equal luminance (Fig. 2a and b). But, for line separations of less than 10' of arc, when the luminance of I was higher T seemed to be displaced towards I (Fig. 2c). An exploratory experiment revealed that this attraction increases monotonically as the luminance difference between inducing and test line increases. For larger separations of the lines one of the subjects (IR) produced two data points with significant ($P \leq 0.05$) values of repulsive displacement whereas the data of another subject (WG) do not show any effect. Standard errors are of the order of magnitude that is to be expected when using an alignment criterion.

The basic findings of our experiment have been confirmed

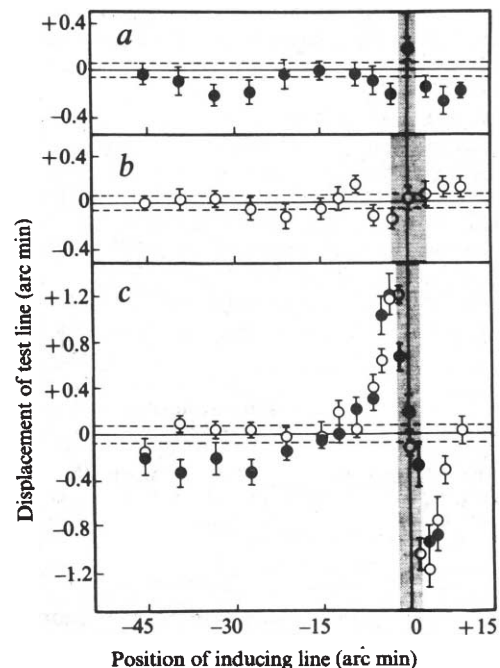


Fig. 2 Changes in apparent position of test line T against distance between T and inducing line I. a, Both lines 0.6 log units above threshold; b, both lines 1.6 log units above threshold; c, test line 0.6 log units, inducing line 1.6 log units above threshold. ●, subject, IR; ○, subject WG. Negative values of displacement mean repulsion at negative values of position (I left of T) and attraction at positive values of position (I right of T). Apparent position of T in absence of I is taken as reference value. Vertical bars $\pm$ s.e. ($n = 12$). Dashed lines indicate $\pm$ s.e. of reference value ($n = 24$). Vertical shaded areas denote where the lines could not be resolved.

for two other subjects. To investigate possible influences of the configuration of the comparison stimulus we also performed measurements using a vernier alignment criterion. The vernier line had a length of 1° and was positioned 0.5° below T. The results of this experiment were indistinguishable from those of the main experiment.

Purely attractive displacements such as those observed in our experiment are readily explained by the model of Blakemore and colleagues, if it is assumed that only excitatory components interact for the assessment of position. But an appreciable shift occurs only if the individual distributions differ considerably in height. The lower peak is then displaced toward the larger one, provided that the separation of the lines is not too large. To account qualitatively for the observed range of interactions (about 10'), it must be assumed that the spread of excitatory activity is wider than the centre of sensitivity functions that are derived from threshold measurement³.

Though there are no inhibitory interactions in the assessment

of position, two lines separated by several minutes of arc clearly inhibit each other. A control experiment using the same apparatus showed that both subthreshold and superthreshold inducing lines raise the threshold for the detection of a test line located 6' away. This suggests that lateral inhibition participates in the neural analysis of contrast (for example, threshold detection) but not in that of position. Thus we are led to the conclusion that the detection of line stimuli and the neural evaluation of their position are performed separately in the human visual system. This receives support from the finding⁵ that the accuracy for positional judgments is an order of magnitude less than the accuracy for shape or orientation discrimination, for which virtually all the information available at the retina is used.

Hubel and Wiesel^{6,7} advanced the idea of an hierarchical organisation of the visual system suggesting a convergence of cortical simple cells on complex cells whose positional selectivity is poor. Psychophysical line detecting mechanisms may be correlated with the activity of monocularly driven simple cells³. If the hierarchical model is correct, the tapping of positional information before the convergence of the output of these cells on complex cells would be necessary to prevent the loss of that information.

Our data are not easy to reconcile with the general conclusion of Ganz¹ that parallel contours repel each other when they are brought close together. By this principle Ganz explained an important class of geometrical illusions (for example, Poggendorff, Hering, Zoellner). Our results do not support this hypothesis as we found no displacement at all for parallel lines of equal brightness.

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- ¹ Ganz, L., *Psychol. Rev.*, **73**, 128 (1966).
- ² Blakemore, C., Carpenter, R. H. S., and Georgeson, M. A., *Nature*, **228**, 37 (1970).
- ³ Rentschler, I., and Fiorentini, A., *Vision Res.*, **14**, 1467 (1974).
- ⁴ Kulikowski, J. J., and King-Smith, P. E., *Vision Res.*, **13**, 1455 (1973).
- ⁵ Andrews, D. P., Webb, J. M., and Miller, D. T., *Vision Res.*, **14**, 757 (1974).
- ⁶ Hubel, D. H., and Wiesel, T. N., *J. Physiol., Lond.*, **160**, 106 (1962).
- ⁷ Hubel, D. H., and Wiesel, T. N., *J. Physiol., Lond.*, **195**, 215 (1968).

Retinotectal mismatch: a serendipitous experimental result

THERE is increasing evidence to indicate that neurones are capable of making connections with sites other than those with which they normally connect¹⁻⁵. During an experiment to test the effect on the retinotectal projection of upsetting the normal sequence of innervation of the growing optic tectum by a growing eye in *Xenopus*⁶, we observed, unexpectedly, a result in one animal which could shed some light on a current controversy concerning the manner in which the formation of selective synaptic connections may be controlled. Because this result was fortuitous and because it is difficult to replicate the exact experimental situation we report the result here.

The right eye had been removed at stage 29 (ref. 7), before the eye had differentiated; and the animal grew up with only one eye, the left, which innervated the right optic tectum. Two months after metamorphosis the left optic nerve was exposed and cut in the region where the optic chiasma would have been in a normal animal. The optic fibres were then deflected to feed directly into the left optic tectum by placing a small piece of Millipore filter in a midline position. The filter was successful in forcing fibres from the whole eye to innervate the ipsilateral (left) tectum, but it did not

prevent some optic fibres from regrowing to the contralateral (right) tectum. In most cases fibres from the whole eye grew directly to both tecta, as shown by electrophysiological mapping of the retinotectal projection 6 months after the deflection of the optic nerve.

In one animal, however, a most intriguing result was obtained (Fig. 1). The projection from the left eye to the left tectum showed the whole nasotemporal extent of the superior visual field covering the dorsal tectal surface—the map was essentially a normal contralateral map, now occurring on the ipsilateral tectum because of the deflection of the optic fibres into this tectum. The same left eye also projected directly to the right optic tectum but in this case the field projection involved only the temporal half of the superior visual field; yet this half-field projected across the whole extent of the dorsal surface of the right tectum. Fibres from only the nasal half of the retina of the left eye seem to have surmounted the millipore barrier and established contact with the right tectum. These nasal retinal fibres (equivalent to the temporal field) in the normal animal occupy only the caudal portion of the tectum and in this animal they occupied only the caudal area of the left tectum. Yet, fibres from this same nasal retina spread their connections across the entire rostrocaudal extent of the right tectum.

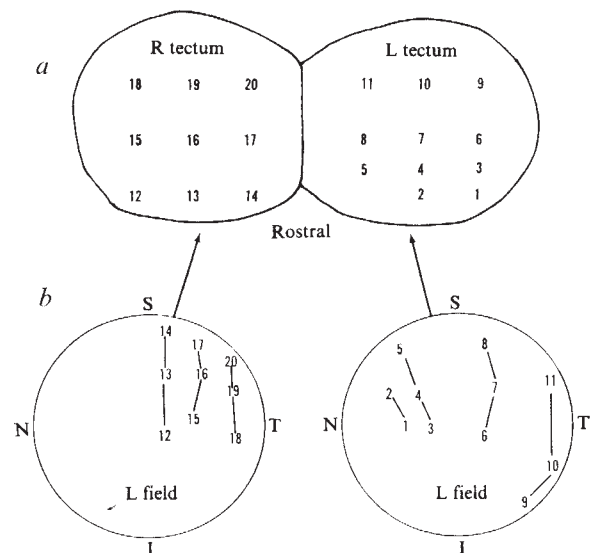


Fig. 1 The projection of the left visual field on to the right and left optic tectum. *a*, The two tecta seen from above; *b*, perimetric charts of the left visual field. The animal should be considered as sitting behind the chart, which covers the visual field from the fixation point out to 100° radially. The numbers on the tectal diagrams represent electrode positions and the numbers on the perimetric chart indicate corresponding field positions. S, Superior; I, inferior; N, nasal; T, temporal.

There have been two different explanations offered to account for previous experimental situations in which half of the retina had been observed to spread its projection over the entire tectal surface⁸⁻¹². One suggestion was that following the operation the nature of the remaining half retina changes and the cell labels which are believed to be responsible for selective neuronal connections^{13,14} are altered. The range of such labels normally associated with a whole retina may thus come to reside in the remaining half retina and this could be the reason that fibres from such a partial retina spread over a whole tectum. An alternative view^{12,15} of the manner in which neurones interconnect argues that the molecular interactions between neurones are essentially competitive, such that the presynaptic fibres compete for the available postsynaptic space.

On this view the fibres from a half retina might be expected to innervate a complete tectum even though the cell labels in the half retina had not changed.

We have here a situation where a half retina, which, because it is half of a known normal eye, should have undergone no reorganisation of its cellular labels, innervates a tectum and covers its whole rostrocaudal extent. This finding constitutes support for the competitive variant of the neuronal specificity theory, in which cellular labels do not, of themselves, determine the tectal site at which a retinal fibre will synapse, but rather fix the relative position within the pre- and postsynaptic arrays that the fibre will adopt. We conclude, therefore, that half a retina which undergoes no reorganisation of its neuronal specificity labels may yet spread its connections in order over an entire tectal surface. Our suggestion that specified neuronal arrays produce selective neuronal connections by a process of competition seems to be supported.

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- ¹ Langley, J. N., *J. Physiol., Lond.*, **22**, 215 (1897).
- ² Mark, R. F., *Br. med. Bull.*, **30**, 122 (1974).
- ³ Raisman, G., *Brain Res.*, **14**, 25 (1969).
- ⁴ Jansen, J. K. S., Lomo, T., Nicolaysen, K., and Westgaard, R. H., *Science*, **181**, 559-561 (1973).
- ⁵ Steward, O., Cotman, C. W., and Lynch, G. S., *Expl Brain Res.*, **20**, 45 (1974).
- ⁶ Feldman, J., Gaze, R. M., and Keating, M. J., *Expl Brain Res.*, **14**, 16 (1971).
- ⁷ Nieuwkoop, P. D. and Faber, J., *Normal Table of Xenopus laevis* (Daudin), (North Holland, Amsterdam, 1967).
- ⁸ Yoon, M., *Am. Zool.*, **12**, 106 (1972).
- ⁹ Gaze, R. M., Jacobson, M., and Székely, G., *J. Physiol., Lond.*, **165**, 484 (1963).
- ¹⁰ Gaze, R. M., Jacobson, M., and Székely, G., *J. Physiol., Lond.*, **176**, 409 (1965).
- ¹¹ Gaze, R. M., Keating, M. J., Székely, G., and Beazley, L., *Proc. R. Soc.*, **B175**, 407 (1970).
- ¹² Straznicki, K., Gaze, R. M., and Keating, M. J., *J. Embryol. exp. Morph.*, **26**, 523 (1971).
- ¹³ Sperry, R. W., *J. comp. Neurol.*, **79**, 33 (1943).
- ¹⁴ Sperry, R. W., in *Handbook of Experimental Psychology* (edit. by Stevens, S. S.), 236-280 (Wiley, New York, 1951).
- ¹⁵ Gaze, R. M., and Keating, M. J., *Nature*, **237**, 375 (1972).

Re-establishment of functional connections by regenerating central adrenergic and cholinergic axons

AFTER lesion of their axons, central monoamine-containing neurones have a high capacity for regenerative sprouting¹⁻⁴. This is observed after a mechanical or electrolytic lesion in the brain or the spinal cord as a development of numerous newly-formed axon sprouts extending from the proximal ends of the lesioned axons. These regenerating sprouts can grow to considerable distances, for example, along myelinated fibre tracts and intracerebral vessels¹⁻³. After selective destruction of the serotonin-containing neurones in the central nervous system (CNS), induced by the neurotoxic drug 5,6-dihydroxytryptamine, the serotonin axons regenerate efficiently and in these favourable conditions it has been possible to demonstrate that lesioned central axons can grow back to their original sites of termination⁴.

With the fluorescence histochemical technique the remarkable regenerative capacity of adult central monoamine neurones is also revealed when lesioned noradrenergic neurones are allowed to 'reinnervate' a transplant of peripheral tissue placed in the brain or spinal cord^{2,3}. In this experimental situation the regenerating central noradrenergic sprouts will reconstitute a terminal pattern in the surviving parts of the grafted tissue that closely mimics its original peripheral noradrenergic innervation. Interestingly, this property does not seem to be unique for the noradrenergic neurones in the brain, in that also presumed central cholin-

ergic neurones have recently been found to have very similar regenerative characteristics (our unpublished observations).

Although the regenerated terminal networks are long lasting, microscopical techniques have not yielded any information as to whether the regenerated central axons reform functional synaptic contacts. We have investigated this question in transplants of the portal vein 'reinnervated' by central noradrenergic and cholinergic neurones in the rat brain. We now report that the regenerated central axons form functional connections with the smooth muscle cells in the transplant. To our knowledge this is the first time a re-establishment of functional connections has been demonstrated after axonal regeneration in the adult mammalian brain.

The experiments were carried out on adult female Sprague-Dawley rats (180-200 g body weight at the time of transplantation). Homologous transplantations of the portal vein to the caudal diencephalon were performed by free hand as described previously for transplantations of other tissues^{3,5}. All recipient animals were subjected to cranial sympathectomy through bilateral extirpation of the superior cervical ganglia to exclude any possible interference by peripheral sympathetic fibres in the transplant. Sections of the portal vein, about 7-8 mm long, were opened by a longitudinal cut and the whole or half of the circumference was used. The portal strip thus obtained was placed vertically in the caudal diencephalon of the recipient rat, extending dorsally through the hippocampus and the corpus callosum³. Transplants of the iris which were placed in this position have previously been found to be invaded by regenerating central noradrenaline (NA)-containing and acetylcholinesterase (AChE)-positive fibres (ref. 3 and our unpublished work).

At 25-30 d after operation, the animals were killed by decapitation under light ether anaesthesia. The transplant was removed from the brain and mounted for combined isometric recordings and electrical field stimulation in a 50 ml organ bath as described previously^{8,9}. The buffer was a modified, oxygenated Krebs solution kept at 38°C throughout the experiment. After an accommodation period of 15-30 min, during which the spontaneous myogenic activity of the transplant was registered, the contractile responses to electrical field stimulation and to exogenous application of neurotransmitters (NA and acetylcholine (ACh)) were studied. Field stimulation was done with square wave impulses of 15 V amplitude between the electrodes (5 mm apart), 1.0 ms duration, and 6 or 10 Hz. The stimulation was applied for 1 min periods at intervals not less than 5 min. NA or ACh was added to the bath to a concentration of 10⁻⁶ M, and was washed out after 1-2 min. Spontaneous activity was allowed to recover between the responses to drugs or field stimulation. The effects of NA and field stimulation were tested before and after applications of the α -adrenergic blocker phenoxybenzamine (PBZ) (Dibenzylamine; Smith, Kline and French; 10⁻⁶ M) and this was followed by a similar second sequence of experiments comprising exposure to ACh and field stimulation before and after the application of atropine sulphate (10⁻⁶ M). On some of the investigated transplants the experiment was done in the reverse order, that is, starting with the cholinergic receptor blockade, followed by the adrenergic blockade.

The regeneration of NA-containing and AChE-positive nerves into the portal transplants was investigated microscopically with the Falck-Hillarp fluorescence method¹⁰ and then with the Holmstedt¹¹ modification of the Koelle method for AChE, respectively. In the AChE-staining, incubation time was 4-6 h and Mipaflox (4 $\times$ 10⁻⁶ M) was used as inhibitor of nonspecific cholinesterases. The histochemistry was carried out on serial sagittal sections of portal vein transplants *in situ*. The isolated transplants used in the field stimulation experiments were subsequently pro-

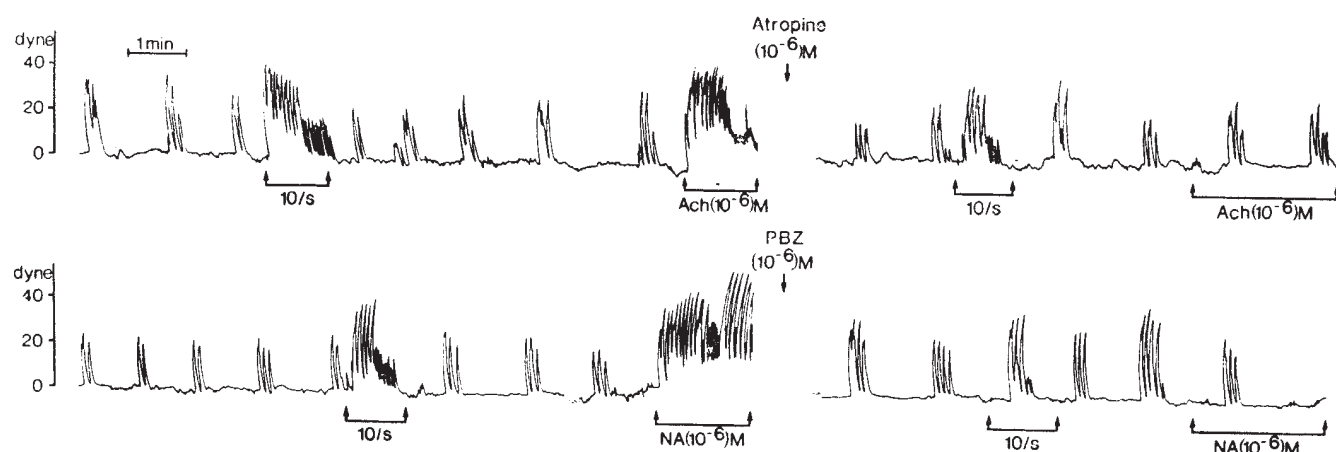


Fig. 1 Spontaneous activity and responses to field stimulation and drug application in a strip of portal vein transplanted to the region of the hippocampus and the caudal diencephalon, 30 d survival. Atropine reduces the response to field stimulation and eliminates completely the effect of ACh. The remaining, atropine-resistant component of the electrical response and the excitatory action of NA are abolished by PBZ. We conclude that in this specimen both cholinergic and adrenergic central neurones have established functional connections with the smooth muscle.

cessed for fluorescence histochemistry of adrenergic nerves.

In its position in the caudal diencephalon and the hippocampus, the portal vein transplant was invaded by regenerating fibres from the locus coeruleus NA axons, running in the so-called dorsal tegmental bundle^{3,6,7} and by AChE-positive fibres (Fig. 2). The principal source of the AChE-positive fibres was from lesioned axons running in the alveus, cingulum and the supracallosal striae, belonging to the septo-hippocampal AChE-positive pathways¹² (Fig. 2c). There is now much evidence¹³⁻¹⁵ that this projection system is cholinergic. A contribution by peripheral AChE-positive parasympathetic fibres was only observed when the transplant reached all the way down to the interpeduncular fossa. In such transplants the parasympathetic fibres of the pial vessels¹⁶ were seen to have grown into the ventral part of the portal strip. In transplants that ended about 1.5 mm or more dorsal to the fossa no such ingrowth of peripheral cholinergic axons could be detected.

In vitro, the normal portal vein shows spontaneous rhythmic contractions with a frequency of about 1–3 min⁻¹ and a strength of about 500 dyne^{8,9}. Transmural stimulation elicits excitatory contractile responses that are blocked by PBZ and by previous sympathetic denervation^{9,17-19}. Exogenous NA and ACh are both excitatory on the myogenic activity of the portal vein, and these actions are totally blocked by α -adrenoreceptor blockers and atropine, respectively^{9,19}.

A total of 17 portal vein transplants were tested *in vitro* and of these 16 showed a good survival as judged by, first, their spontaneous myogenic activity, and, second, their contraction responses to NA and ACh administered to the bath. Some of the transplants had a regular, rhythmic spontaneous activity much resembling that of the intact portal vein (Fig. 1), but the contractions were weaker (about 20–110 dyne). The periods of activity were often more prolonged than normal, and occurred with a frequency of 0.5–2 min⁻¹. The remaining preparations had a more disordered spontaneous activity, without clear-cut periods of rest. Most of the specimens retained their spontaneous activity throughout the experiment (usually 2–2.5 h). Field stimulation at 6 to 10 Hz evoked excitatory contractile responses in 11 of the spontaneously active portal transplants. Optimally, the stimulation-induced contractions were of the same magnitude as those elicited by 10⁻⁶ M NA or ACh in the bath (Fig. 1).

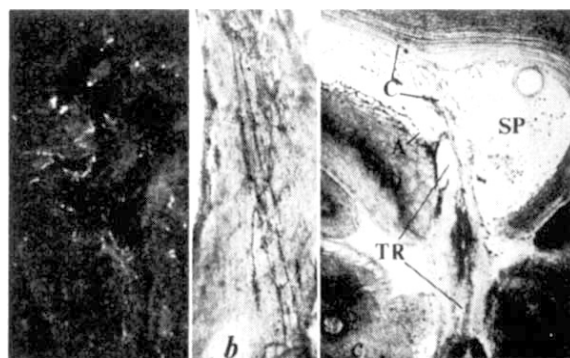
PBZ was used as the first blocker in 8 of the 11 preparations. The response to field stimulation was abolished in two, was reduced in four and remained virtually unaffected in two muscles of this group. Atropine was given to four of the six muscles with PBZ-resistant responses and in all

these the effect of field stimulation was completely abolished (Fig. 1). Three muscles received atropine as the first blocker. The response to field stimulation was abolished in one and remained in two of these preparations; these atropine-resistant responses were eliminated after PBZ. The results thus show that all responses to field stimulation could be abolished by PBZ and atropine, individually or in combination, indicating functional innervation of the muscles by adrenergic and/or cholinergic fibres. It may be pointed out that PBZ and atropine had no effect on the spontaneous myogenic activity but abolished specifically the responses to NA (10⁻⁶ M) and ACh (10⁻⁶ M), respectively. The efficiency of the receptor blockade was always tested by application of NA and ACh in these concentrations.

The fluorescence microscopical analysis of the *in vitro* stimulated transplants showed a good correlation between the PBZ-sensitive contractile responses to field stimulation and the presence of NA-containing fibres in the transplant. In the transplants showing no, or only very weak, spontaneous activities there occurred areas of necrosis, bleeding, or scar formation, and they were often poorly reinnervated from the brain.

The combined histochemical and physiological data provide strong evidence for functional neuromuscular connections being formed by the regenerated central adrenergic

Fig. 2 Reinnervation of portal vein transplants by central adrenergic and AChE-positive fibres, 25–30 d survival. *a*, Regenerated central NA-containing fibres in the muscle layer of the portal vein transplant, as seen in a transverse section of the transplant *in situ* (Falck–Hillarp method; $\times 75$); *b*, regenerated central AChE-positive fibres in a squash preparation of the isolated transplant ($\times 75$); *c*, sagittal section through the transplant in its position in the hippocampus (Hi) and the dorsal diencephalon (Di). AChE-positive fibres are seen to grow into the transplant (TR) from the alveus (A) and the cingulum (C). SP denotes the splenium of the corpus callosum. Rostral is to the right ($\times 16$).



and cholinergic axonal terminal networks in the transplanted portal strips. It is well established^{9,19,20} that transmural stimulation of the type used in the present study selectively excites the nerves in the tissue, and, therefore that the contractile responses are neurally mediated. The effects of the receptor blockers observed in this study are consistent with this view and support the conclusion that the responses registered in the portal CNS transplants were due to the release of NA and ACh from the regenerated central fibres, acting on the smooth muscle receptors.

The adrenergic reinnervation was primarily accomplished by the locus coeruleus neurones. The characteristics of the neurotransmission restituted by the central adrenergic fibres in the transplant were quite similar to those of the normal portal vein^{17,18} or those of the portal vein reinnervated by peripheral sympathetic fibres²¹. Moreover, the efficacy of the central reinnervation was high in terms of the magnitude of the postsynaptic responses to neural stimulation. In fact, in the successful specimens they were as large as those evoked by a high concentration of exogenous NA.

Although it was not possible to remove surgically the intracranial parasympathetic innervation—as was done with the sympathetic one—it seems clear that at least in the more dorsally situated transplants the cholinergic neuroeffector responses were exclusively, or almost exclusively, mediated by regenerated central fibres, originating primarily from lesioned axons of the septo-hippocampal pathways. Interestingly, such cholinergic responses (remaining after α -adrenoreceptor blockade and being abolished by atropine) have not been detected in the normal rat portal vein although muscarinic cholinergic receptors are present^{9,17,18}. It therefore seems possible that in its location in the brain normally non-innervated cholinergic receptors will become reinnervated by the regenerating central cholinergic neurones, giving rise to a heterologous innervation similar to that described after cholinergic reinnervation of the cat nictitating membrane^{22–24}.

The very limited restoration of function in adult mammals after brain damage is often explained by an insufficient regenerative capacity of central neurones and by their inability to re-establish functional connections^{25,26}. An important implication of the current studies on the reinnervation of peripheral transplants in the CNS would be that—when given the same growth conditions as in the peripheral nervous system—at least some neurone systems in the mammalian CNS will show an entirely adequate regenerative capacity resulting in a restitution of normal neurotransmission in the denervated tissue.

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- 1 Katzman, R., Björklund, A., Owman, Ch., Stenevi, U., and West, K. A., *Brain Res.*, **25**, 579–596 (1971).
- 2 Björklund, A., Katzman, R., Stenevi, U., and West, K. A., *Brain Res.*, **31**, 21–33 (1971).
- 3 Björklund, A., and Stenevi, U., *Brain Res.*, **31**, 1–20 (1971).
- 4 Björklund, A., Nobin, A., and Stenevi, U., *Brain Res.*, **50**, 214–220 (1973).
- 5 Stenevi, U., Bjerre, B., Björklund, A., and Mobley, W., *Brain Res.*, **69**, 217–234 (1974).
- 6 Ungerstedt, U., *Acta physiol. scand.*, supplement 367, 1–48 (1971).
- 7 Lindvall, O., and Björklund, A., *Acta physiol. scand.*, supplement 412, 1–48 (1974).
- 8 Axelsson, J., Wahlström, B., Johansson, B., and Jonsson, O., *Circulation Res.*, **21**, 609–618 (1967).
- 9 Johansson, B., Ljung, B., Malmfors, T., and Olson, L., *Acta physiol. scand.*, supplement 349, 5–16 (1970).
- 10 Björklund, A., Falck, B., and Owman, Ch., in *Methods of Investigative and Diagnostic Endocrinology* (edit. by Berson, S. A.): *The Thyroid and Biogenic Amines* (edit. by Rall, J. E., and Kopin, I. J.), 318–368 (North-Holland, Amsterdam, 1972).
- 11 Holmstedt, B., *Acta physiol. scand.*, **40**, 331–337 (1957).

- 12 Lewis, P. R., and Shute, C. C. D., *Brain*, **90**, 521–540 (1967).
- 13 Lewis, P. R., Shute, C. C. D., and Silver, A., *J. Physiol., Lond.*, **191**, 215–224 (1967).
- 14 Fonnum, F., *J. Neurochem.*, **17**, 1029–1037 (1970).
- 15 Kuhar, M. J., Sethy, V. H., Roth, R. H., and Aghajanian, G. K., *J. Neurochem.*, **20**, 581–593 (1973).
- 16 Edvinsson, L., Nielsen, K. C., Owman, Ch. and Spörng, B., *Z. Zellforsch.*, **134**, 311–325 (1972).
- 17 Häggendal, J., Johansson, B., Jonason, J., and Ljung, B., *Acta physiol. scand.*, supplement 349, 17–32 (1970).
- 18 Häggendal, J., Johansson, B., Jonason, J., and Ljung, B., *J. Pharm. Pharmacol.*, **24**, 161–164 (1972).
- 19 Holman, M. E., Kasby, C. B., Suthers, M. B., and Wilson, J. A. F., *J. Physiol., Lond.*, **196**, 111–132 (1968).
- 20 Paterson, G., *J. Pharm. Pharmacol.*, **17**, 341–349 (1965).
- 21 Olson, L., and Malmfors, T., *Acta physiol. scand.*, supplement 348, 1–112 (1970).
- 22 Vera, C. L., Vail, J. D., and Lucio, J. V., *J. Neurophysiol.*, **20**, 365–373 (1957).
- 23 Lennon, A. M., Vera, C. L., Rex, A. L., and Lucio, J. V., *J. Neurophysiol.*, **30**, 1523–1530 (1967).
- 24 Koslow, S. H., Giacobini, E., Kerpel-Fronius, S., and Olson, L., *J. Pharmac. exp. Ther.*, **180**, 664–671 (1972).
- 25 *Regeneration in the Central Nervous System* (edit. by Windle, W. F.) (Thomas, Springfield, 1955).
- 26 Clemente, C. D., *Int. Rev. Neurobiol.*, **6**, 257–301 (1964).

Differentiation of opiate agonist and antagonist receptor binding by protein modifying reagents

OPIATE antagonists differ from opiates only by the replacement of the N-methyl substituent with an N-allyl, N-cyclopropylmethyl or related group. Antagonists can reverse the pharmacological effects of opiates and so are important in the treatment of opiate overdose. Drugs that combine agonist and antagonist activities have potential as relatively nonaddicting analgesics. Although their mutual pharmacological antagonism indicates that they act at the same binding site, pharmacological discrepancies have prompted speculation that agonist and antagonist binding sites are separate^{1,2}. Biochemical study of receptor binding^{3–16} has shown that although opiate agonists and antagonists compete for the same receptor^{3,4}, there are differences in the way they interact with them^{7,10}. Physiological concentrations of sodium enhance the binding of ³H-antagonists but reduce that of ³H-agonists. Sodium diminishes the ability of pure agonists to inhibit binding of ³H-naloxone to the receptor, but has little influence on inhibitory effects of pure antagonists and intermediate effects on those of combination agonist-antagonist drugs. The molecular basis for these differences, however, is unclear. As a first step in this direction we now report that binding of opiate receptors is much more sensitive to degradation by protein reagents which are known to modify sulphhydryl groups than is binding by opiate antagonists. This suggests that distinct binding sites exist for agonists and antagonists, although they may both be on the same receptor.

Brains, minus cerebella, from male Sprague-Dawley rats (180–220 g) were homogenised in standard buffer (50 mM Tris-HCl, pH 7.7 at 25° C) and centrifuged at 49,500g for 15 min; the pellets were resuspended in 100 volumes of standard buffer, incubated at 37° C for 30 min, recentrifuged and resuspended in 100 volumes of standard buffer. Incubation at 37° C increases opiate receptor binding about 80% (G.W.P. and S.H.S., in preparation). The homogenates were then treated with the appropriate reagent, washed by centrifugation at 49,500g for 15 min and assayed with 100 mM NaCl and the appropriate ³H-opiate at 25° C for 30 min in the presence and absence of 1 μ M levallorphan, unless otherwise stated. Samples were filtered and counted as before^{3,6}. All results are the mean of triplicate determinations and are expressed as specific opiate receptor binding, representing total binding minus binding in the presence of 1 μ M levallorphan.

Treatment of rat brain membrane preparations with iodoacetamide (20 mM) markedly reduced the specific receptor binding of the ³H-opiate agonists oxymorphone, levorphanol and dihydromorphone but not of the ³H-opiate antagonists naloxone and levallorphan (Table 1). To ensure that these effects were not due to interactions of iodoac-

Table 1 Effect of iodoacetamide on receptor binding of ^3H -opiate agonists and antagonists

Opiate	Stereospecific opiate binding (c.p.m.)		
	Control	Iodoacetamide Treated	% Change
Antagonists			
^3H -naloxone	1,040	1,092	+ 5
^3H -levallorphan	1,551	1,672	+ 8
Agonists			
^3H -oxymorphone	719	401	-44
^3H -levorphanol	1,288	827	-36
^3H -dihydromorphine	1,871	878	-53

Rat brains were homogenised in 20 volumes of standard Tris buffer and centrifuged at 50,000*g* for 15 min. The pellet was resuspended in 100 volumes of standard Tris buffer and equal volumes were incubated in the presence and absence of 20 mM iodoacetamide for 20 min at 25°C. The homogenates were then centrifuged as before, resuspended in their original volumes and assayed with either (+)-3-hydroxy-N-allylmorphinan or (-)-3-hydroxy-N-allylmorphinan (levallorphan) at 200 nM and the appropriate ^3H -opiate. Samples were filtered and counted as described in the text. The following concentrations of ^3H -opiates were used: 1.7 nM ^3H -naloxone; 2.8 nM ^3H -levallorphan; 3.7 nM ^3H -oxymorphone; 4 nM ^3H -levorphanol, and 0.7 nM ^3H -dihydromorphine.

tamide with ^3H -drugs themselves, the experiments were performed with membrane preparations presumably washed free of iodoacetamide. To explore the possibility that residual iodoacetamide might have reacted with the labelled ligands, thin-layer chromatograms in three solvent systems were prepared for all five ^3H -opiates after incubation with 100 mM iodoacetamide for 20 min at 25°C. Under these conditions there were no detectable changes in the relative fronts of any of the ^3H -drugs. Treatment with iodoacetamide did not alter the amount of radioactivity at the origin as would be expected for the products of an iodoacetamide-opiate reaction. Moreover, if the effects of iodoacetamide were due to reaction of the ^3H -opiates with residual reagent, an extra wash should have removed more iodoacetamide and lessened the inhibition of binding. Membranes subjected to a second centrifugation and resuspension showed no lessening of inhibition. Thus, the effects of iodoacetamide are irreversible and not due to chemical modification of the labelled ligands.

To examine the specificity and sensitivity of the differential degradation of agonist and antagonist binding, we compared the effects of various concentrations of iodoacetamide, mercuriacetate, iodoacetic acid, *p*-aminophenylmercuric acetate, *p*-chloromercuribenzoate and *N*-ethylmaleimide on opiate receptor binding of ^3H -naloxone and ^3H -dihydromorphine (Table 2). At 5 mM, iodoacetamide maximally inhibited binding of ^3H -dihydromorphine by about 90%; in other experiments 0.1 mM iodoacetamide lowered it by 25%. By contrast, binding of ^3H -naloxone showed little sensitivity to iodoacetamide between 5 mM and 100 mM. *N*-ethylmaleimide similarly discriminated between binding of ^3H -naloxone and ^3H -dihydromorphine. Concentrations of 0.1 mM to 3 mM *N*-ethylmaleimide maximally inhibited ^3H -dihydromorphine binding 90–100% and as little as 10 μM *N*-ethylmaleimide lowered it by 66%. Only a 30% decrease in ^3H -naloxone binding could be detected at 0.3 mM *N*-ethylmaleimide with 35% and 60% reductions in binding at 1 and 3 mM *N*-ethylmaleimide respectively. *p*-Chloromercuribenzoate also discriminated between agonist and antagonist binding. While 20 μM *p*-chloromercuribenzoate lowered ^3H -dihydromorphine binding by about 85%, it decreased that of ^3H -naloxone by only 45%. Differential effects on the binding of agonists and antagonists were also found for *p*-aminophenylmercuric acetate and mercuriacetate, although their effects were not as dramatic. There was a clear-cut separation of the effects of *p*-aminophenylmercuric acetate at 20 μM on binding of ^3H -naloxone and ^3H -dihydromorphine with a 75% decrease in ^3H -dihydro-

morphine binding and only a 45% decrease in ^3H -naloxone binding.

Unlike the other reagents, iodoacetic acid at 1–25 mM did not differentiate between agonists and antagonists, having little effect on the binding of either ^3H -dihydromorphine or ^3H -naloxone.

All the reagents which inhibit opiate receptor binding as described here are known to affect sulphhydryl groups, but can affect other amino acid residues^{17–23}, and so we investigated whether their action derives from an effect on sulphhydryl groups. Brain homogenates were incubated with 10 μM or 50 μM *p*-aminophenylmercuric acetate for 10 min at 25°C and centrifuged. The pellets were resuspended in the original volume of buffer, divided into aliquots and reacted with β -mercaptoethanol (10 mM and 20 mM for aliquots treated 10 μM and 50 μM *p*-aminophenylmercuric acetate, respectively) or water. Samples were again centrifuged and the pellets resuspended in their original volume of buffer and assayed with ^3H -dihydromorphine. The binding was abolished in samples treated only with 10 μM and 50 μM *p*-aminophenylmercuric acetate. In samples exposed to 10 and 20 mM β -mercaptoethanol after the *p*-aminophenylmercuric acetate (10 and 50 μM), however, there was only a 45% and 65% reduction in binding. The ability of a sulphhydryl-containing compound to reverse the lowering of opiate receptor binding suggests that *p*-aminophenylmercuric acetate decreases binding of ^3H -dihydromorphine by an action on sulphhydryl groups associated with the opiate receptor.

In preliminary studies to determine whether protein reagents modify the opiate binding site itself, we attempted to protect the binding site by preincubating with opiates before treatment with 10 μM iodoacetamide. The binding

Table 2 Effect of protein modifying reagents on receptor binding of ^3H -naloxone and ^3H -dihydromorphine

Reagent	^3H -Naloxone binding (% control)	^3H -Dihydromorphine binding (% control)
Iodoacetamide		
5 mM	86	9
25 mM	77	15
50 mM	80	16
100 mM	78	0
N-Ethylmaleimide		
0.1 mM	85	7
0.3 mM	71	9
1.0 mM	65	16
3.0 mM	40	3
p-Aminophenylmercuric acetate		
10 μM	83	71
20 μM	54	26
40 μM	10	6
p-Chloromercuribenzoate		
20 μM	53	16
40 μM	34	13
Iodoacetate		
1 mM	100	90
3 mM	87	81
5 mM	98	89
25 mM	86	80
Mercuriacetate		
10 μM	79	42
100 μM	39	2

Rat brains were homogenised and prepared as described in the text. All reagents were dissolved in water immediately before use except for *p*-aminophenylmercuric acetate, mercuriacetate and *p*-chloromercuribenzoate, which were dissolved in dimethylsulphoxide. Dimethylsulphoxide in the concentrations used has no effect on binding. ^3H -naloxone was present at 1 nM and ^3H -dihydromorphine at 0.6 nM. All binding assays were performed in the presence of 100 mM NaCl. All experiments were replicated at least twice. Each value is the mean of triplicate determinations which varied less than 8%.

site for ^3H -dihydromorphine can be protected from the influence of iodoacetamide by preincubation with naloxone, oxymorphone, morphine and nalorphine at $0.2\ \mu\text{M}$ (our work in preparation).

Our findings thus demonstrate chemical differences in binding sites on the opiate receptor for agonists and antagonists. Sulphydryl groups seem to be important components for differentiating the interactions of opiate agonists and antagonists with the opiate receptor.

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- 1 Martin, W. R., *Pharmac. Rev.*, **19**, 463–521 (1967).
- 2 Martin, W. R., Gorodetzky, C. W., and Thompson, W. O., *Agonist and antagonist actions of narcotic analgesic drugs* (edit. by Kosterlitz, H. W., Collier, H. O., and Villareal, J. E.), 30–40 (University Park Press, Baltimore, 1973).
- 3 Pert, C. B., and Snyder, S. H., *Science*, **179**, 1011–1014 (1973).
- 4 Pert, C. B., and Snyder, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2243–2247 (1973).
- 5 Pasternak, G. W., and Snyder, S. H., *Molec. Pharmacol.*, **10**, 183–193 (1974).
- 6 Pasternak, G. W., and Snyder, S. H., *Nature* (in the press).
- 7 Pert, C. B., Pasternak, G. W., and Snyder, S. H., *Science*, **182**, 1359–1361 (1973).
- 8 Kuhar, M. J., Pert, C. B., and Snyder, S. H., *Nature*, **245**, 447–450 (1973).
- 9 Pert, C. B., Snowman, A., and Snyder, S. H., *Brain Res.*, **70**, 184–188 (1974).
- 10 Pert, C. B., and Snyder, S. H., *Molec. Pharmacol.*, **10**, 868–879 (1974).
- 11 Terenius, L., *Acta Pharmac. Toxicol.*, **33**, 377–384 (1973).
- 12 Terenius, L., *Acta Pharmac. Toxicol.*, **34**, 88–91 (1974).
- 13 Simon, E. J., Hiller, J. M., and Edelman, I., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1947–1949 (1973).
- 14 Wong, D. T., and Horng, J. S., *Life Sci.*, **13**, 1543–1556 (1973).
- 15 Klee, W. A., and Streety, R. A., *Nature*, **248**, 61–63 (1974).
- 16 Hitzemann, R. J., and Loh, H. H., *Third Ann. Soc. Neuroscience Mtg.*, Abstr., 350 (1973).
- 17 Crestfield, A. M., Stein, W. H., and Moore, S., *J. biol. Chem.*, **238**, 2421–2428 (1963).
- 18 Smyth, D. G., Nagamatsu, A., and Fruton, J. S., *J. Am. chem. Soc.*, **82**, 4600–4604 (1960).
- 19 Riggs, A., *J. biol. Chem.*, **236**, 1948–1954 (1961).
- 20 Crestfield, A. M., Stein, W. H., and Moore, S., *J. biol. Chem.*, **238**, 2413–2420 (1963).
- 21 Cohen, L. A., *The Enzymes* (edit. by Boyer, P.), **1**, 148–211 (Academic, New York, 1970).
- 22 Vallee, B. L., and Riordan, J. F., *Ann. Rev. Biochem.*, **38**, 733–794 (1969).
- 23 Cohen, L. A., *Ann. Rev. Biochem.*, **37**, 695–726 (1968).

Requirements for bursting pacemaker potential activity in molluscan neurones

THE mechanisms underlying endogenous rhythmical electrical activity of some molluscan neurones are not completely understood. Their membranes have several significant properties: (1) rapidly inactivating Na^+ and Ca^{2+} conductances associated with spikes^{1–5}; (2) voltage-dependent K^+ conductances (G_K) (delayed^{1,6} and anomalous rectification^{3,7}); and (3) a negative slope region (NSR) in the steady-state, voltage clamp current-voltage (I - V) curve^{8,9}. Although (1) is unnecessary for bursting pacemaker potentials (BPPs)⁴ the relationship of (2) and (3) to the generation of BPPs has not been elucidated completely. We present here evidence that the NSR of the I - V curve is due to a voltage-dependent Na^+ conductance (G_{Na}) which inactivates incompletely and that BPPs depend on this conductance coupled to cyclical changes in G_K .

In a typical experiment fused ganglia were isolated from the land snail (*Otala lactea*) or the sea hare (*Aplysia californica*), fixed in a chamber and perfused with saline (in mM, for *Aplysia*: 500 NaCl, 10 KCl, 50 MgCl_2 , 10 CaCl_2 ,

and 10 Tris-Cl, pH 7.8; for *Otala*: 100 NaCl, 4 KCl, 10 CaCl_2 , 5 MgCl_2 , 5 Tris-Cl, pH 7.8) at 20 – 21°C . Cell 11 of *Otala*³ or R15 of *Aplysia*² which normally generate BPPs were penetrated with two microelectrodes (3 M KCl or 7.5 M CsCl). Conventional methods were used to record the cell's electrical activity and voltage clamp its membrane potential. One microelectrode monitored V_m while the other carried current. The settling time of the system was about 1 ms for voltage steps up to 50 mV. Clamping current was measured by an operational amplifier that held the bath at ground.

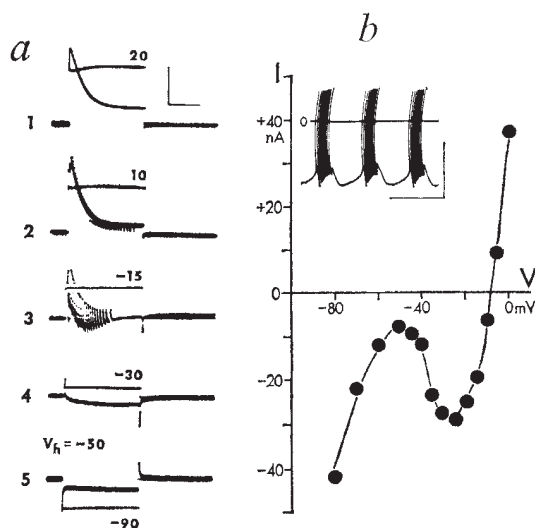


Fig. 1 Data from cell R15 of *Aplysia californica*. *a*, From voltage clamp. Voltage (thin lines) and current (thick lines) traces for 5-s pulse commands from holding potential (V_{holding} , V_h) of -50 mV to potentials indicated by numbers adjacent to voltage trace. Traces begin with voltage and current traces superimposed. Positive-going potentials and (outward) currents are displayed upward. Calibration: 45 mV for voltage traces, 500 nA (trace 1), 200 nA (trace 2), 100 nA (trace 3) and 50 nA (traces 4 and 5) for current traces; 2.5 s. See text for details. *b*, I - V curve from voltage clamp data shown in (*a*) (see text for details). Inset shows spontaneous BPP oscillations in the unclamped cell. Calibrations 50 mV, 25 s. In this and subsequent figures zero membrane potential is indicated by a horizontal line marked 0.

The steady-state current recorded from cells generating BPPs under voltage clamp are always inward (I_{in}) between -60 and -30 mV , the normal BPP range^{8,9}. The membrane was clamped between -60 and -30 mV to a potential which required the least holding I_{in} . Voltage step commands were applied (4–5 s) and the currents required to clamp the membrane at various potentials recorded. Four currents were identified: (1) an inward somatic spike current (not shown due to the slow time base); (2) a slowly decaying outward K^+ current (delayed rectification) for large depolarising steps (Fig. 1*a*, traces 1–3), (3) a voltage-dependent increase in I_{in} for steps between -45 and -30 mV , (trace 4) and (4) a time-dependent increase in inward current during large hyperpolarising commands (anomalous rectification) (trace 5). There were also rapid biphasic currents (traces 2 and 3) representing spikes in unclamped portions of the cell's axon.

I - V curves were constructed by measuring the minimum, slow changing current (least positive or most negative) during each voltage step at times greater than 1 s after the change in V_m and plotting this plus the holding current against the command voltage (Fig. 1*b*). Inward spike currents were excluded. Sometimes the slowly changing current was partially obscured by axon spike currents (for example, trace 3, where the minimum current was estimated at 2 s during the command step). The I - V curves were N-shaped (Fig. 1*b*) and the net, quasi-steady state current of cells generating BPPs rarely, if ever, became outward at

a V_m more negative than -30 mV. Qualitatively similar I - V curves were obtained in all cells generating BPPs. I_{in} from cell 11 was small but could be increased by replacing $[Ca^{2+}]_o$ and by iontophoresing Cs^+ ions intracellularly. This allowed quantitative analysis of the I_{in} and NSR.

The ionic mechanisms underlying the voltage-dependent I_{in} were assessed by stepwise replacement of most of Na^+ with equimolar amounts of $Tris^+$, Li^+ or sucrose. Reductions of $[Na^+]_o$ decreased the size of the I_{in} evoked at a given V_m (-30 mV in Fig. 2b) and of the NSR (Fig. 2c). All substitutions for $[Na^+]_o$ gave similar results (Fig. 2d); I_{in} at a given V_m varied linearly with $[Na^+]_o$. Reducing either the $[Cl^-]_o$ with SO_4^{2-} or methanesulphonate anions or the $[K^+]_o$ did not alter the I_{in} . These results suggest that the increased I_{in} evoked over the -50 to -30 mV V_m range is a Na current (I_{Na}) due primarily to a voltage-dependent Na^+ conductance (G_{Na}). This G_{Na} activates rapidly with depolarisation since the I_{in} develops quickly. The early decline in the I_{in} may represent some inactivation of G_{Na} but the persistence of I_{in} for the duration of a 5 s pulse indicates that G_{Na} does not inactivate completely. On returning V_m to the holding V_m , the inward tail currents relax within seconds, suggesting that G_{Na} inactivates quickly with hyperpolarisation.

The I - V properties of this persistent G_{Na} were evaluated

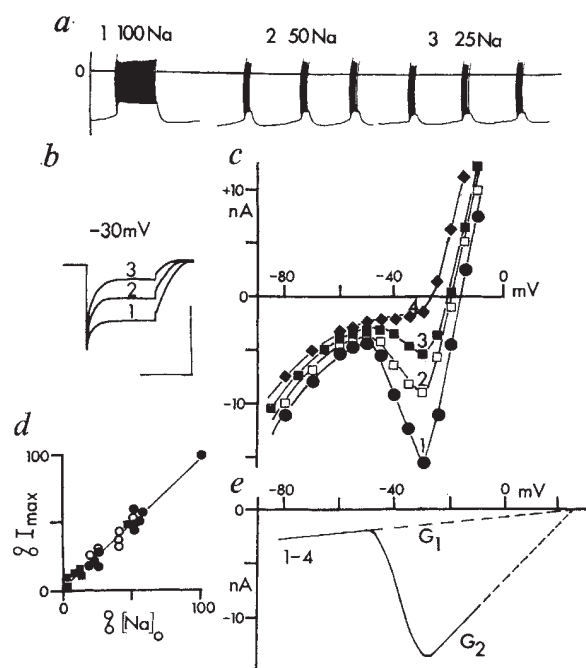


Fig. 2 Effects of altering $[Na^+]_o$ on membrane properties of BPP neurones. Data in (a-c) and (e) from cell 11 of *Otala lactea*. d contains data from both cell 11 and cell R15. Cs^+ ions were iontophoresed intracellularly by means of a microelectrode in cell 11 before measurements. In (a-c) data labelled 1 (or $\bullet$), 2 (or $\square$) and 3 (or $\blacksquare$) were obtained when the $[Na^+]_o$ was, respectively, 100, 50 and 25 mM (Na^+ replaced with $Tris^+$). Other extracellular ions were kept constant (10 mM $SrCl_2$, 4 mM KCl). a, Effect of reducing $[Na^+]_o$ on the BPP; b, line traces of voltage-clamp currents recorded in pulsing the membrane from holding of -50 mV to -30 mV for 5 s in different $[Na^+]_o$ s; c, plots of voltage-clamp I - V curves. The curve linked by diamonds was taken in 25 mM $NaCl$, 80 mM $SrCl_2$, 4 mM KCl and 5 mM $Tris-Cl$. V_h always -50 mV; d, the clamp current, at -30 mV, as a percentage of current required to clamp the membrane at -30 mV in maximum $[Na^+]_o$ against the percentage of the maximum $[Na^+]_o$. $[Na^+]_o$ replacements were Li^+ ($\blacksquare$), $Tris^+$ ($\bullet$) and sucrose ($\circ$). e, Curve $\bullet$ minus curve $\blacktriangle$ of (c), see text. The solid parts of the curve are from the data in (c). The dashed lines are straight-line extrapolations, see text. Calibrations: 90 mV, 25 s (a); 12.5 nA, 4 s (b).

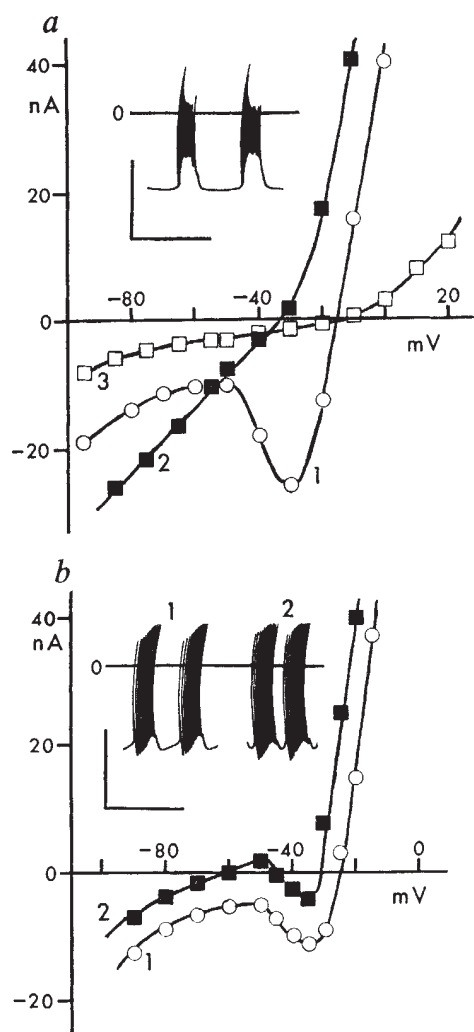


Fig. 3 Effects of extracellular ionic alteration on BPP activity and on membrane properties in cell 11 of *Otala lactea*. In all experiments extracellular $NaCl$ (100 mM) and $Tris-Cl$ (5 mM) were held constant. a, I - V ($\circ$) the medium also contained 10 mM $SrCl$ and 4 mM KCl and the cell had been injected with Cs^+ . Inset: BPPs under such conditions. $\blacksquare$, KCl was replaced with 8 mM $CsCl$; $\square$, the cell was returned to 4 mM KCl and the $SrCl_2$ replaced with 10 mM $CoCl_2$; V_h for all curves in a = -50 mV. b, For $\circ$ the medium contained 10 mM $CaCl_2$ and 4 mM KCl , $V_h = -50$ mV. Inset 1: spontaneous BPP activity; $\blacksquare$ the KCl was reduced to 1 mM and 5 mM $MgCl_2$ was added to the medium. The cell was silent at -60 mV but BPP activity could be elicited by injecting a steady 5 nA depolarising current through an intracellular microelectrode (inset 2). Calibration: 50 mV, 25 s.

by subtracting the I - V curve obtained when the persistent I_{Na} was assumed to be zero from that under control conditions (Fig. 2c, curve 1). The former conditions existed in a solution containing 25 mM $[Na^+]_o$ and 80 mM $[Sr^{2+}]_o$ which abolished the NSR of the curve without changing other measured currents (Fig. 2c, curve 4). The subtraction yields a curve (Fig. 2e) that indicates this persistent G_{Na} has voltage-independent and voltage-dependent components, which extrapolate to a reversal potential of about $+25$ mV, near the peak of the Na-dependent spike of this cell (Fig. 2a, part 1). These data suggest that the persistent I_{in} required to clamp the membrane at potentials in the NSR of the I - V curve is carried by Na^+ ions and that the NSR of the steady-state I - V curve is mainly due to a voltage-dependent, incompletely or non-inactivating G_{Na} . This persistent G_{Na} differs from the G_{Na} associated with spikes: (1) its thresh-

hold for activation is more negative (Fig. 1a); (2) inactivation is incomplete or absent (Figs 1a and 2b); (3) tetrodotoxin-sensitivity is absent⁴; (4) Li^+ cannot substitute for Na^+ in otherwise normal saline and (5) it is very temperature sensitive⁸.

Our data also indicate that the amplitude of the BPP (the difference between the trough and crest of a BPP) correlated with the magnitude of the persistent I_{Na} (Fig. 2a and d). Qualitative changes in these two variables (BPP, I_{Na}) resulted from other ionic manipulations: both were abolished by replacing $[\text{K}^+]_0$ with $[\text{Cs}^+]_0$ which markedly increased membrane conductance (Fig. 3a). When unclamped, the cell depolarised, fired repetitively and would not generate BPPs with hyperpolarising current (not shown). Both variables were also lost by replacing $[\text{Sr}^{2+}]_0$ with $[\text{Co}^{2+}]_0$ (Fig. 3a); again, BPP activity was not restored by extrinsic current. Others have shown correlations between the NSR and BPP activity when they were altered by temperature changes⁸ and pharmacologically⁹. While these data suggest that a persistent, voltage-dependent G_{Na} is required for BPP activity, it is not sufficient. Application of sufficient hyperpolarising or depolarising current can prevent BPPs^{3,4}, leading either to silence or repetitive firing. V_m behaviour changed similarly when the bathing solution was altered. When MgCl_2 was added and KCl was reduced the cell hyperpolarised to -60 mV and became silent; however, injection of a steady depolarisation current restored BPPs (Fig. 3b, inset 2). Conversely, after excessive Cs^+ injections, the cell shown in Fig. 3a depolarised to -15 mV and generated BPPs only with steady hyperpolarising current (Fig. 3a, inset). Under voltage clamp, both cells had a persistent G_{Na} and the V-axis intersected the I - V curves near the resting V_m of the unclamped membrane (-60 mV and -15 mV, respectively) (Fig. 3b, curve 2 and Fig. 3a, curve 1). Thus, these procedures resulted in a stable potential on a positive slope of the I - V curve and zero current was required to clamp V_m . Cells with spontaneous BPPs did not have a resting potential; the intersection

of the V-axis with their I - V curves was therefore, not a stable potential.

Another proposed requirement for BPP activity is a G_{K} with appropriate voltage- and time-dependencies^{3,12,13}. To study this contribution, V_m was clamped to -50 mV at different times during the BPP cycle and the resulting currents recorded (Fig. 4b, traces 1-4). (The record in Fig. 4a was not taken when the cell was clamped, but only indicates the times during the cycle when V_m was clamped.) The tail current following return of V_m to -50 mV from a 5 s command to -15 mV is also shown (Fig. 4b, trace 5). This tail current is most likely due to a G_{K} (refs 1, 6) for times longer than 5 s all currents decay exponentially with a time constant of about 16 s. If these are K^+ currents, the zero time intercepts of the curves in Fig. 4c (traces 1-4) reflect G_{K} at the onset of the clamp (values listed under $I_{\text{K}}(0)$ in Fig. 4b). G_{K} is minimal at the beginning of the bursting phase of the BPP (time 1), increases during the bursting period (times 2 and 3), becomes maximal at its end and decreases with time as the V_m slowly depolarises (times 4 to 1), as previously suggested^{3,12,13}.

In summary, two membrane properties are associated with BPP activity: (1) a G_{Na} with voltage-independent and voltage-dependent components which inactivates incompletely; (2) a voltage-dependent G_{K} with slow kinetics in the BPP range of V_m . Additional prerequisites include adequate membrane resistance and the absence of a steady-state electromotive force.

A possible mechanism of the interaction of these properties in producing spontaneous BPP activity is as follows. Let the V_m begin at its most hyperpolarised point; the slow decline in the voltage-dependent G_{K} which brought V_m to this point, along with the voltage-independent component of the persistent G_{Na} , depolarises V_m . When V_m reaches threshold for the voltage-dependent component of the persistent G_{Na} , it activates quickly and V_m depolarises rapidly. V_m remains depolarised from lack of inactivation of G_{Na} . This allows a burst of spikes and activates the voltage-dependent G_{K} which eventually generates sufficient hyperpolarising force to lead to the post-burst hyperpolarisation of V_m . This hyperpolarisation occurs rapidly since the voltage-dependent component of G_{Na} turns off quickly, and another BPP cycle begins. This description thus extends the hypothesis that pacemaking activity is due to a high resting G_{Na} and the cycling G_{K} . It also indicates the importance of the NSR in the I - V curve since this region is due to the voltage-dependent G_{Na} which inactivates incompletely.

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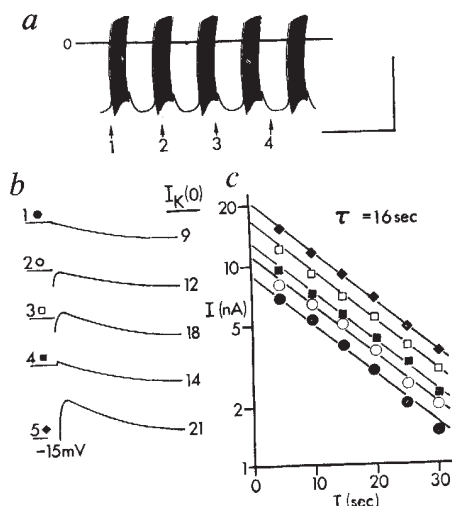
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- Alving, B. O., *J. gen. Physiol.*, **54**, 512 (1969).
- Carpenter, D., and Gunn, R., *J. Cell Physiol.*, **75**, 121 (1970).
- Gainer, H., *Brain Res.*, **39**, 403 (1972).
- Strumwasser, F., *The Physiologist*, **16**, 9 (1973).
- Stinnakre, J., and Tauc, L., *Nature new Biol.*, **242**, 113 (1973).
- Eaton, D. C., *J. Physiol., Lond.*, **224**, 421 (1972).
- Faber, D. S., and Klee, M., *Nature new Biol.*, **240**, 29 (1972).
- Wachtel, H., and Wilson, W. A., in *Neurobiology of Invertebrates, Mechanisms of Rhythm Regulation*, (edit by Salanki, J.), 59 (Akademiai Kiado, Budapest, 1973).
- David, R. J., Wilson, W. A., and Escudé, A. V., *Brain Res.*, **67**, 549 (1974).
- Frazier, W. T., Kandel, E. R., Kupfermann, I., Waziri, R., and Coggeshall, R. E., *J. Neurophysiol.*, **30**, 1288 (1967).
- Barker, J. L., and Gainer, H., *Nature*, **245**, 462 (1973).
- Carpenter, D. O., in *Neurobiology of invertebrates: Mechanisms of Rhythm Regulation* (edit. by Salanki, J.), 35 (Akademiai Kiado, Budapest, 1973).
- Junge, D., and Stephens, C., *J. Physiol., Lond.*, **235**, 155 (1973).

Fig. 4 Currents recorded by voltage clamping at different phases of the spontaneous BPP cycle. *a*, Arrows indicate times during the BPP cycle when the cell was clamped to -50 mV; *b*, traces 1-4 are line drawings of the currents recorded at the times numbered in (*a*). Zero current indicated by the horizontal lines below the numbers in each trace. Trace 5: the tail current recorded when the cell was returned to V_h (-50 mV) from a 5-s step to -15 mV; *c*, semilog plot of current time for currents shown in (*b*). The slowly declining currents all fit an exponential with a time-constant of decay (τ) of about 16 s. Zero time was the onset of the clamp for 1-4 and the beginning of the tail-current in 5. Zero-time intercepts of each of these curves are listed under $I_{\text{K}}(0)$ in (*b*). Calibration: 60 mV, 30 s in (*a*); 60 nA, 30 s in (*b*).



Selection for diploid cells in suspension cultures of *Haplopappus gracilis*

CHANGES in the number and structure of chromosomes of plant cells cultured *in vitro* are of common occurrence¹, and cell populations devoid of diploid karyotype have been reported²⁻⁴. The chromosome number is known to become variable even in clones derived from single cells^{5,6}. In the case of *Picea glauca* Voss. ($n = 21$) callus cultures, a positive correlation between growth rate and chromosome number was observed⁷, which indicates a selection for polyploid cells. Only diploid cells were, however, observed in mitosis in suspension cultures of *Haplopappus gracilis* (Nutt.) Gray ($n = 2$) (ref. 2), *Medicago sativa* L. ($n = 16$) (ref. 8) and *Crepis capillaris* L. Wallr. ($n = 3$) (ref. 9). This could result from polyploid cells dividing less frequently than diploid cells, as diploid and polyploid cells differ physiologically. A strong selection for diploid cells was observed¹⁰ in *Vicia hajastana* Grossh. ($n = 5$) suspension cultures initiated from mature seeds. It therefore seemed likely that cell cultures of other plant species with a low chromosome number, $n = 7$ or less, would show a selection for diploid cells.

Callus cultures were initiated from hypocotyl segments of 3-d-old seedlings of *H. gracilis* on agar B5 medium¹¹ containing 4.5×10^{-6} M 2,4-dichlorophenoxyacetic acid (2,4-D). The calli were kept in a culture room at constant temperature (27–28°C) and light (2,000 lx). Subculturing was carried out every 3 weeks. After 122 d in culture, some cells were transferred to liquid B5 medium with 2,4-D at the same concentration. The suspension cultures were subcultured every 3 d, and were agitated by a gyratory shaker at 150 r.p.m. The cytological technique was essentially that of Singh *et al.*¹⁰.

An increase in the frequency of aneuploid cells was observed after 94 d in culture; a drastic decrease subsequently occurred. The frequency of diploid cells increased consistently whereas that of tetraploid cells steadily declined (Table 1). Anaphase analyses revealed a number of anomalies, such as bridges, fragments, unequal distribution of chromatids and laggards in low frequencies (1–3%). Chromosomes with altered mor-

phology were also observed at metaphase. These anomalies could have been induced by 2,4-D, or some other factor, present in the culture medium, and would lead to the production of aneuploid cells from diploid and polyploid cells. Furthermore, endoreduplication is likely to occur in plant cells cultured *in vitro*^{12,13}. The increase in the frequency of diploid cells under such a condition, therefore, clearly shows a strong selection for such cells. A similar selection for diploid cells was observed in suspension cultures of *H. ravenii* ($n = 4$), a closely related species, initiated from stem segments¹⁴.

The cytogenetic behaviour of *H. gracilis* cells has been investigated previously^{2,15-18}. In some studies, a systematic analysis of the changes in the frequencies of various karyotypes during the *in vitro* culture was not made^{2,16,17}, but where such an analysis was made^{15,18}, an increase in the frequency of polyploid cells was observed. Since agar media were used^{15,18}, these findings do not necessarily contradict the results reported here. An increase in the frequency of polyploid cells was observed when *H. gracilis* cells were cultured on agar B5 medium¹⁹. Only diploid cells were observed in a suspension culture of *H. gracilis*, which was interpreted to indicate a selection for diploid cells².

The distribution of the two chromosomes of *H. gracilis* in the aneuploid cells was non-random; chromosome I was present more often than expected. In fact, almost all of the cells with five chromosomes had an extra chromosome I. A similar non-random distribution of chromosomes has been observed in suspension cultures of *V. hajastana*¹⁰. Since both chromosome I and II seem to be equally susceptible to mitotic irregularities, it seems that cells possessing an extra chromosome I were at a selective advantage over those possessing an extra chromosome II.

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- Partanen, C. R., *Int. Rev. Cytol.*, **15**, 215–243 (1963).
- Kao, K. N., Miller, R. A., Gamborg, O. L., and Harvey, B. L., *Can. J. Genet. Cytol.*, **12**, 297–301 (1970).
- Torrey, J. G., *Physiologia Plant.*, **20**, 265–275 (1967).
- Singh, B. D., *Caryologia* (in the press).
- Cooper, L. S., Cooper, D. C., Hildebrandt, A. C., and Riker, A. J., *Am. J. Bot.*, **51**, 284–290 (1964).
- Muir, W. H., *Proc. int. Conf. Plant Tissue Culture* (edit. by White, P. R., and Grove, A. R.), 485–490 (McCutchan, Berkeley, 1965).
- DeTorok, D., and Roderick, T. H., *Cancer Res.*, **22**, 174–181 (1962).
- Clement, W. H., *Am. J. Bot.*, **51**, 670 (1964).
- Reinert, J., and Kuster, H. J., *Z. Pflanzenphysiol.*, **54**, 213–222 (1966).
- Singh, B. D., Harvey, B. L., Kao, K. N., and Miller, R. A., *Can. J. Genet. Cytol.*, **14**, 65–70 (1972).
- Gamborg, O. L., Miller, R. A., and Ojima, K., *Expl Cell Res.*, **50**, 151–158 (1968).
- Bennici, A., Buiatti, M., and D'Amato, F., *Chromosoma*, **24**, 194–201 (1968).
- D'Amato, F., *Proc. int. Conf. Plant Tissue Culture*, (edit. by White, P. R., and Grove, A. R.), 449–462 (McCutchan, Berkeley, 1965).
- Singh, B. D., *Caryologia* (in the press).
- Bennici, A., Buiatti, M., D'Amato, F., and Pagliai, M., *Les Cultures de Tissus de Plantes*, 245–250 (Colloques Internationaux CNRS No. 193, Paris, 1970).
- Mitra, J., and Steward, F. C., *Am. J. Bot.*, **48**, 358–368 (1961).
- Reinert, J., and Torrey, J. G., *Naturwissenschaften*, **48**, 132–133 (1961).
- Shamina, Z. B., *Proc. Symp. on the Mutational Process* (edit. by Landa, Z.), 377–380 (Academia, Prague, 1966).
- Singh, B. D., and Harvey, B. L., *Cytologia* (in the press).

Lack of permeability of mouse placenta to maternal and foetal cells

IN man, foetal leukocytes circulate in the maternal blood in most pregnancies and are occasionally detected up to 1 yr after delivery¹⁻³. On the other hand, maternal white blood corpuscles have seldom been found in the foetal

Table 1 Frequencies (%) of different cell types in two suspension cultures (A and B) of *H. gracilis*

Chromosome class	Days in culture				
	7	14	94	175	258
Hypodiploid (<4)					
Culture A	3.0	1.1	5.4	0.8	—
B	2.0	2.1	2.9	0.7	1.4
Mean	2.5	1.6	4.3	0.7	0.7
Diploid (2n = 4)					
Culture A	52.0	58.7	60.8	82.4	92.0
B	55.0	55.2	57.1	86.7	93.8
Mean	53.5	56.9	59.2	84.7	92.9
Hyperdiploid (5)					
Culture A	4.0	1.1	13.8	7.6	2.5
B	1.0	1.0	22.9	5.3	1.4
Mean	2.5	1.1	17.9	6.5	1.9
Hypotetraploid (6 and 7)					
Culture A	6.0	9.8	3.1	—	0.6
B	3.0	8.3	3.8	0.7	1.4
Mean	4.5	9.1	3.4	0.4	1.0
Tetraploid (4n = 8)					
Culture A	35.0	28.3	13.8	9.2	4.9
B	32.0	29.2	9.5	6.7	2.1
Mean	33.5	28.7	11.9	7.8	3.6
Hypertetraploid (>8 <16)					
Culture A	—	1.1	2.3	—	—
B	1.0	2.1	3.8	—	—
Mean	0.5	1.5	3.0	—	—
Octaploid (8n = 16)					
Culture A	2.0	—	0.8	—	—
B	4.0	2.1	—	—	—
Mean	3.0	1.1	0.4	—	—
No of cells observed					
Culture A	100	92	130	131	163
B	100	96	105	150	146
Total	200	188	235	281	309

circulation⁶⁻⁸. The reason for this paradoxical persistence of potentially histoincompatible cells in the mother's circulation is unknown. The fact that paternally-derived foetal HL-A antigens are difficult to demonstrate in cord blood samples⁹ suggests that the factors determining incompatibility in the foetal cells are either masked by blocking antibodies or are not expressed¹⁰.

In the mouse, information exists only on the passage of blood cells from mother to foetus¹¹⁻¹⁶. Both radioactive labelling¹¹⁻¹³, and cytogenetic markers^{12-14,16}, have been used to differentiate between foetal and maternal cells. The data are, however, inconsistent¹¹⁻¹⁶, probably resulting from the pitfalls of the methods used.

To overcome methodological difficulties, pregnancies resulting from laboratory mouse $\times$ tobacco mouse matings were studied. The laboratory mouse has 40 acrocentric chromosomes, whereas the tobacco mouse has only 26 chromosomes, 14 of which are metacentric¹⁷. Since the hybrid has 33 chromosomes¹⁸, seven of which are metacentric, a mitosis from a foetus of such a cross can be distinguished without difficulty from a parental mitosis.

The passage of lymphocytes from foetus to mother was studied in phytohaemagglutinin (PHA) or leukoagglutinin (LA) cultures of blood from female laboratory mice mated with male tobacco mice. Blood samples were taken between the second and third weeks of pregnancy (in ten instances), or immediately after delivery (in five instances), and cultured as described¹⁹. Chromosome preparations were made by conventional methods²⁰ and stained with Giemsa; 100-1,000 mitoses were studied from each animal.

Transmission of blood cells in the opposite direction, from mother to foetus, was studied in identical matings. Ten newborn mice (two from each of five litters) were killed and chromosome preparations made from liver, spleen, bone marrow and thymus¹², and stained with Giemsa. A total of 10,000 mitotic cells was studied.

Table 1 Mitotic studies on cultures of lymphocytes from female laboratory mice mated with male tobacco mice

	No. of animals	No. of cells	No. of foetal cells
During pregnancy	10	4,995	0
After delivery	5	4,096	0
Total	15	9,091	0

PHA or LA cultures were carried out between weeks 2 and 3 of pregnancy in ten animals, and immediately after delivery in five animals.

The results of chromosome studies on maternal lymphocyte cultures are presented in Table 1. Foetal mitoses were never found in these cultures; neither was there any evidence of transmission of blood cells from mother to foetus.

The results of Tuffrey *et al.*^{12,13}, however, indicate transmission of maternal cells into the foetus. These workers used the T₆ chromosome marker in a syngeneic system to distinguish foetal mitoses from maternal mitoses and found maternal cells in up to 33% of mice which had developed in the uterus of foster mothers after transplantation at the blastocyst stage. Later, using the same marker, they found maternal cells in the offspring after normal pregnancies. Others^{14,16} using the T₆ marker, have been unable to confirm these data, and suggest that differences between mouse strains or misinterpretation of the T₆ chromosome are possible reasons for these discrepancies. The main limitation of these studies is that only mitoses, and not interphase cells, have been studied. Negative results might be due to inability of foetal and maternal cells crossing the mouse placenta to enter mitotic division in such experiments. We have also examined mitotic cells.

Radioactive labelling has been used to mark maternal blood cells in the circulation of the offspring^{11,15}. One group¹¹ was able to demonstrate transplacental passage of red blood corpuscles in a few pregnancies, and the other¹⁵

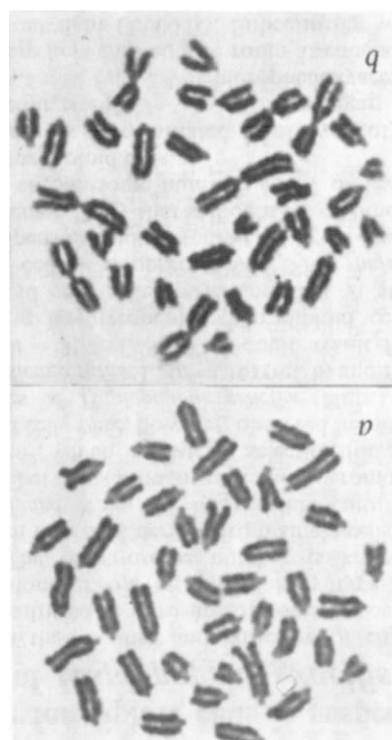


Fig. 1 *a*, Cultured lymphocyte of a laboratory mouse in mitosis. The cell has 40 acrocentric chromosomes. *b*, A mitosis from a chromosome preparation of liver from a laboratory mouse $\times$ tobacco mouse hybrid. The cell has 33 chromosomes, seven of which are metacentric.

found maternal erythrocytes and leukocytes in the blood of most foetuses. The method is open to question, however, as passage of free isotope molecules across the placenta and uptake of these by foetal cells cannot be excluded.

The human placenta seems to be permeable to blood cells, and foreign cells invade the circulation of both the foetus and the mother often without causing any clinical symptoms¹⁻⁸ and so it may seem surprising that this is not so in the mouse. Reasons may be that the placental structure of the two species differ; differences in the number of foetuses, time of gestation or in the validity of the methods used to study the phenomenon may also be responsible. In man, the methods seem to be adequate and the results convincing, but in the mouse suitable markers are lacking. One limitation is the lack of markers for mitotic studies, the other the failure to devise a method for distinguishing between foetal and maternal cells during interphase. We have overcome the first problem but not the second. We have not found any evidence for the transplacental passage of blood cells in the mouse, but the results must be regarded with caution until markers are found by which foetal and maternal cells can be distinguished from each other at interphase.

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¹ Walknowska, J., Conte, F. A., and Grumbach, M., *Lancet*, i, 1119 (1969).

² de Grouchy, J., and Trebuchet, C., *Ann. Génét.*, 14, 133 (1971).

- ³ Schröder, J., and de la Chapelle, A., *Blood*, 39, 153 (1972).
⁴ Schröder, J., Tiilikainen, A., and de la Chapelle, A., *Transplantation*, 17, 346 (1974).
⁵ Grosset, L., Barrelet, V., and Odartchenko, N., *Am. J. Obstet. Gynec.* (in the press).
⁶ Turner, J. H., Wald, N., and Quinlivan, M. D., *Am. J. Obstet. Gynec.*, 95, 831 (1966).
⁷ Olding, L., *Acta Paed. scand.*, 61, 73 (1972).
⁸ Schröder, J., *Scand. J. Immunol.*, 3, 369 (1974).
⁹ Tiilikainen, A., Schröder, J., and de la Chapelle, A., *Transplantation*, 17, 355 (1974).
¹⁰ Hellström, K. E., Hellström, J., and Bergheden, C., *Nature*, 208, 458 (1965).
¹¹ Finegold, M., and Michie, D., *J. Embryol. exp. Morph.*, 9, 618 (1961).
¹² Tuffrey, M., Bishun, N. P., and Barnes, R. D., *Nature*, 221, 1029 (1969).
¹³ Tuffrey, M., Bishun, N. P., and Barnes, R. D., *Nature*, 224, 701 (1969).
¹⁴ Billington, W. D., *Nature*, 224, 704 (1969).
¹⁵ Barnes, R. D., and Tuffrey, M., *Adv. Biosci.*, 6, 457 (1970).
¹⁶ Seller, M. J., *Nature*, 225, 1254 (1970).
¹⁷ Gropp, A., Tettgenborn, U., and von Lehmann, E., *Cytogenetics*, 9, 9 (1970).
¹⁸ Zech, L., Evans, E. P., Ford, C. E., and Gropp, A., *Exp. Cell Res.*, 70, 263 (1972).
¹⁹ Hayry, P., Virolainen, M., and Defendi, V., *Proc. Soc. exp. Biol. Med.*, 133, 637 (1970).
²⁰ Hungerford, D. A., *Stain Technol.*, 40, 333 (1965).

Cytochalasin B, the blood platelet release reaction and cyclic GMP

CYTOCHALASIN B (CB) affects a wide range of cellular processes that appear to be contractile in nature¹. The molecular mechanisms responsible for these effects are uncertain, though it is known that CB interferes with microfilament function¹ and can block transport of glucose across cell membranes^{2,3}. Secretion by exocytosis has been shown to be inhibited by CB in some cell types⁴⁻⁸ but to be potentiated by CB in others⁹⁻¹². We describe here the effects of CB on the release reaction of blood platelets induced by collagen fibres, a process in which the contents of both amine storage granules (5-HT, ADP, ATP) and lysosomes are secreted¹³ and which leads to platelet aggregation mediated by the ADP released¹⁴. Although previous studies have shown that CB inhibits various platelet activities including clot retraction¹⁵⁻¹⁷ and platelet aggregation^{16,17}, we now find that lower concentrations of CB markedly potentiate both the secretory activities of the platelet and release-dependent aggregation. Because collagen-induced platelet aggregation is associated with an increase in intracellular cyclic GMP¹⁸, a compound which potentiates secretion by some cells^{19,20}, and

because it has been suggested that CB may stimulate leukotaxis by increasing cyclic GMP²¹, we have studied the relationship between the platelet release reaction and platelet cyclic GMP levels in the presence and absence of CB. The results show that CB does not potentiate the release reaction through any direct effect on cyclic GMP levels.

Experiments were performed either with human heparinised platelet-rich plasma (PRP) adjusted to contain 470,000 platelets μl^{-1} (ref. 22) or with a suspension of washed human platelets (560,000 μl^{-1}) suspended in Tyrode's solution containing 0.35% bovine serum albumin and apyrase²³. Platelet 5-HT was labelled by preincubating PRP with 1 μM ¹⁴C-5-HT (ref. 22) for 1 h or washed platelets with 2 μM ¹⁴C-5-HT for 20 min during the first wash. Samples (0.85 ml) of labelled PRP or washed platelet suspension were mixed with 2 μl dimethyl sulphoxide $\pm$ CB and 48–128 μl 0.154 M NaCl and were incubated at 37° C for 10 min before addition of further 0.154 M NaCl $\pm$ collagen to give a final volume of 1 ml. Samples were transferred to an apparatus for the turbidometric measurement of platelet aggregation (Payton Associates Ltd., Scarborough, Ontario) 0.5 min or 1 min before the last addition and then stirred continuously. Incubations were usually terminated by addition of 0.1 ml 0.077 M Na₃EDTA and centrifugation for 0.5 min at 12,000g (Eppendorf 3200). ¹⁴C and enzyme activities were measured in the supernatants and in a corresponding volume of PRP or washed platelet suspension in which the platelets were lysed (see Table 1 for methods). Values in the supernatant from a control without CB or collagen were subtracted and the percentage release of platelet constituents calculated. CB did not affect the activities of the enzymes assayed. Bovine collagen (Sigma) was prepared for use by homogenisation in 0.154 M NaCl at 0° C (Polytron homogeniser); coarse fibres were removed by centrifugation (100g, 5 min). The collagen remaining in suspension was determined gravimetrically after high speed centrifugation, washing with water and drying at 110° C.

Preliminary experiments established that 0.2% dimethyl sulphoxide (the solvent for CB) did not affect the release reaction and that CB did not affect the uptake of ¹⁴C-5-HT by platelets. Addition of CB without collagen did not cause

Table 1 Effects of CB on the release of platelet constituents induced by collagen fibres

Experiment 1	Additions		Release of platelet constituents (%)			
	Collagen ($\mu\text{g ml}^{-1}$)	CB ($\mu\text{g ml}^{-1}$)	¹⁴ C-5-HT	β -Glucuronidase	β -N-Acetylglucosaminidase	Lactate dehydrogenase
	0	5	<1	<1	<1	<1
		50	<1	<1	<1	<1
	1	0	1	<1	<1	<1
		1	14	<1	2	<1
		2	17	2	3	<1
		5	30	3	2	<1
		10	44	4	6	<1
		50	26	<1	3	2
	30	0	75	11	17	<1
		1	79	14	31	2
		2	81	21	35	<1
		5	81	21	35	1
		10	80	21	37	3
		50	75	12	35	3
Experiment 2	0	0.1	<1	<1	<1	<1
		10	<1	<1	<1	<1
	6.5	0	9	1	8	<1
		0.03	15	3	8	<1
		0.1	28	5	14	<1
		0.3	23	3	12	<1
		1	2	<1	2	<1
		10	1	<1	<1	<1

PRP was used in experiment 1 and washed platelets in experiment 2. Final concentrations of collagen and CB are given. Incubations were terminated 3 min after addition of collagen. ¹⁴C was counted by liquid scintillation¹⁸. β -Glucuronidase and β -N-acetylglucosaminidase were assayed at 30° C in 50 mM citrate buffer, pH 4.5, containing 0.25% Triton X-100 and 4 mM *p*-nitrophenyl- β -D-glucuronide or *p*-nitrophenyl-N-acetyl- β -D-glucosaminide respectively. After 20 h or 2 h respectively these reactions were stopped with 9 volumes 0.02 N NaOH and the *p*-nitrophenol liberated was measured spectrophotometrically at 405 nm. Lactate dehydrogenase was assayed at 30° C in the presence of 0.1% Triton X-100 using a standard method²⁴.

Table 2 Effects of CB on the platelet ^3H -cyclic GMP levels observed in the presence of weak and strong release stimuli

Additions	Release of ^{14}C -5-HT (%)	^3H -Cyclic GMP d.p.m. per sample	2P
CB	<1	$1,297 \pm 105$	>0.2
Collagen (2 $\mu\text{g ml}^{-1}$)	1	$2,222 \pm 357$	
Collagen (2 $\mu\text{g ml}^{-1}$) + CB	23	$4,761 \pm 465$	<0.02, >0.01
Collagen (14 $\mu\text{g ml}^{-1}$)	48	$4,214 \pm 160$	
Collagen (14 $\mu\text{g ml}^{-1}$) + CB	62	$4,230 \pm 296$	>0.95
Collagen (136 $\mu\text{g ml}^{-1}$)	76	$5,519 \pm 786$	>0.2
Collagen (136 $\mu\text{g ml}^{-1}$) + CB	78	$6,844 \pm 778$	
ADP (2 μM)	2	$4,555 \pm 261$	
ADP (2 μM) + CB	1	$4,919 \pm 159$	>0.2

The final CB concentration was $5 \mu\text{g ml}^{-1}$ when present. Incubations were terminated 2 min after addition of the release stimulus (final concentration given). ^3H -Cyclic GMP values are means $\pm$ s.e.m. from three identically treated samples of PRP. The significance of the effects of CB was determined by unpaired Student's *t* tests.

aggregation or induce the release of platelet constituents. But addition of $1\text{--}20 \mu\text{g CB ml}^{-1}$ to PRP before a threshold amount of collagen markedly increased the extent of platelet aggregation (Fig. 1). This was associated with a several-fold increase in release of ^{14}C -5-HT, suggesting that the enhanced aggregation was mediated by ADP released with the 5-HT. An almost identical potentiation of ^{14}C -5-HT release was observed whether CB was added with the collagen or up to 50 min before. The greatest increase in release was with $10 \mu\text{g CB ml}^{-1}$ in PRP (Fig. 1; Table 1, experiment 1). Despite these effects, CB delayed platelet aggregation and at concentrations greater than $20 \mu\text{g ml}^{-1}$ suppressed the change in platelet shape (upward deflection of

the recording) that normally precedes aggregation (Fig. 1). Higher CB concentrations ($> 40 \mu\text{g ml}^{-1}$) also delayed and inhibited the release reaction. With amounts of collagen that induced complete platelet aggregation only the inhibitory effects of CB on aggregation were seen in turbidometric recordings, even when release of ^{14}C -5-HT was potentiated. Collagen, particularly at high concentrations, also induced the release of the lysosomal enzymes, β -glucuronidase and β -N-acetylglucosaminidase, from platelets in PRP. In the presence of $10 \mu\text{g CB ml}^{-1}$ the amounts released were approximately doubled (Table 1). In no experiment were more than trace amounts of lactate dehydrogenase released, indicating that CB specifically potentiated the release reaction rather than causing a generalised leakage of cell constituents. Very similar results were obtained with washed platelets (Table 1, experiment 2), but the concentration of CB required for maximum potentiation of the release reaction was much lower ($0.1 \mu\text{g ml}^{-1}$). Above $1 \mu\text{g ml}^{-1}$, CB almost completely suppressed both the release of washed platelet constituents and aggregation.

Our finding that low concentrations of CB can potentiate the platelet release reaction suggests that under appropriate conditions other secretory processes reported to be inhibited by CB^{4-8} may be similarly potentiated. In two instances there is already evidence for this^{25,26}. The question arises whether the potentiation and inhibition of the release reaction by CB involve different mechanisms of action at the molecular level. Lin *et al.*²⁷ have reported that bovine platelets have high and low affinity binding sites for CB and that serum reduces binding to both. The dissociation constants of CB at these two classes of binding site in the presence and absence of serum correlate moderately well with the respective concentrations we required for potentiation or inhibition of the release reaction in the human PRP and washed platelet systems. Lin *et al.*²⁷ suggest that their high affinity sites may be associated with hexose transport and the low affinity sites with cellular contractility. Although CB does block glucose metabolism in platelets¹⁷, it seemed unlikely that this could potentiate the release reaction. We therefore investigated the possibility that cyclic GMP might mediate this action of CB.

To permit measurement of changes in platelet cyclic GMP by a prelabelling method¹⁸, $2 \mu\text{M } ^3\text{H}$ -guanine (4 Ci mmol^{-1}) was included with ^{14}C -5-HT in the preincubation step in some experiments with PRP. ^3H -Cyclic GMP was then isolated from incubations stopped by addition of $0.2 \text{ ml } 3 \text{ N HClO}_4$ with unlabelled cyclic GMP¹⁸. Collagen caused a concentration-dependent increase in platelet ^3H -cyclic GMP up to about four times the basal level (Table 2). The ^3H -cyclic GMP level increased rapidly after a short interval at approximately the same time as the most rapid release of ^{14}C -5-HT (Fig. 2). As release could not be measured instantaneously, we could not determine whether or not the increase in ^3H -cyclic GMP preceded the release reaction. After the initial rapid increase, the ^3H -cyclic GMP concentration rose more slowly for at least 3 min, despite cessation of release and some reuptake of

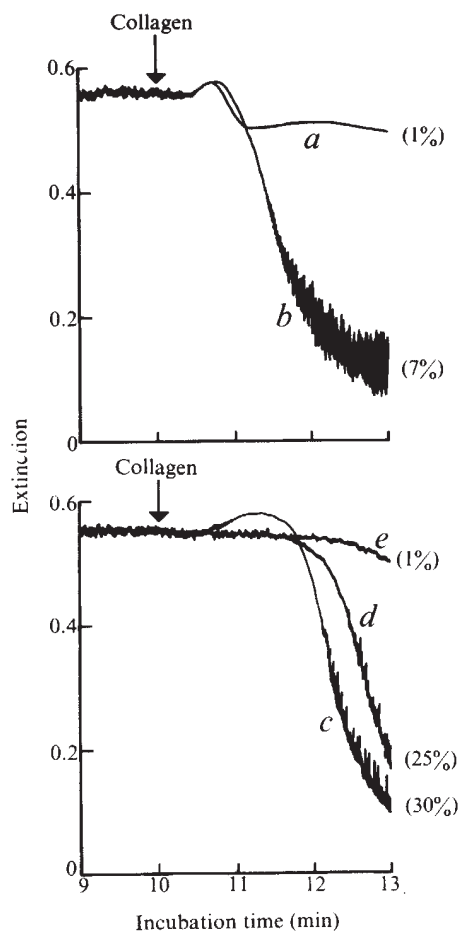


Fig. 1 Effects of CB on platelet aggregation and release of ^{14}C -5-HT induced by collagen. The amount of collagen added (final concentration $2 \mu\text{g ml}^{-1}$) was adjusted so as to cause only slight platelet aggregation in the absence of CB. Incubations were terminated 3 min after addition of collagen. Release of ^{14}C -5-HT (%) at this time is indicated in parentheses. Final CB concentrations ($\mu\text{g ml}^{-1}$) were: a, 0; b, 2; c, 10; d, 20; e, 40.

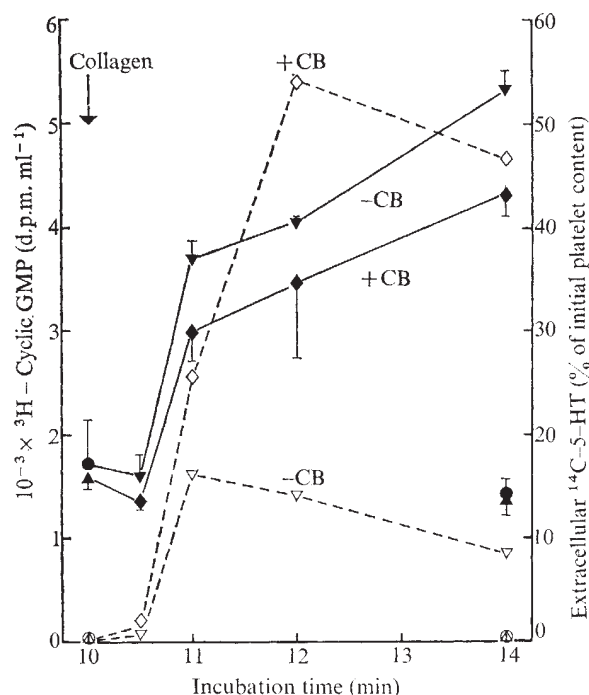


Fig. 2 Platelet ^3H -cyclic GMP levels during release of ^{14}C -5-HT induced by collagen in the presence and absence of CB. Final CB and collagen concentrations were 10 and $5.4 \mu\text{g ml}^{-1}$ respectively when present. ^3H -Cyclic GMP values are means $\pm$ s.e.m. from three identically treated samples of PRP. Closed symbols, ^3H -cyclic GMP (d.p.m.); open symbols, ^{14}C -5-HT released (%). $\circ$, $\bullet$, Controls without CB or collagen; Δ , ∇ , CB alone; $\blacklozenge$, $\diamond$, collagen alone; $\blacklozenge$, $\diamond$, CB and collagen.

^{14}C -5-HT. CB (5 or $10 \mu\text{g ml}^{-1}$) had no statistically significant effect on basal ^3H -cyclic GMP levels or on the increases caused by moderate or high doses of collagen, although it markedly potentiated the release reaction induced by the former (Fig. 2, Table 2). These findings indicate that cyclic GMP does not mediate the potentiation of release by CB. With threshold doses of collagen, however, CB did increase ^3H -cyclic GMP to a highly significant extent (Table 2). This can be explained by the observation that addition of ADP alone also increased ^3H -cyclic GMP (Table 2). Thus, potentiation of the release of ADP by CB probably provided an important extracellular stimulus for the formation of ^3H -cyclic GMP, when little collagen was present. That collagen itself at higher concentrations also stimulates formation of cyclic GMP is indicated by previous observations that inhibition of collagen-induced release and aggregation by aspirin does not block the associated increase in platelet ^3H -cyclic GMP¹⁸. Addition of ADP with or without CB did not cause release of any platelet ^{14}C -5-HT (Table 2). Thus, even if an increase in cyclic GMP is necessary for the release reaction to occur, other factors provided by collagen but not ADP in heparinised PRP are also required. The results indicate that increases in ^3H -cyclic GMP in human platelets may be a relatively nonspecific response to stimulation. Whether platelet aggregation, the release reaction or other platelet activities are facilitated in the presence of increased intracellular cyclic GMP levels remains to be established.

If the potentiation of the release reaction by CB is not mediated by cyclic GMP, what other possibilities exist? It is conceivable that a single class of membrane binding sites could both inhibit glucose transport and facilitate the membrane fusion required for exocytosis²⁸. These membrane sites could be on a protein, possibly platelet actin or myosin, elsewhere associated with the contractile apparatus of the platelet in such a way that their affinity for CB is reduced^{29,30}. Interaction of CB with these latter sites would then account for the inhibitory action of the compound on platelet activities.

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- Wessels, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T. and Yamada, K. M., *Science*, **171**, 135-143 (1971).
- Kletzien, R. F., Perdue, J. F., and Springer, A., *J. biol. Chem.*, **247**, 2964-2966 (1972).
- Estensen, R. D., and Plagemann, P. G. W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1430-1434 (1972).
- Schofield, J. G., *Nature new Biol.*, **234**, 215-216 (1971).
- Thoa, N. B., Wooten, G. F., Axelrod, J., and Kopin, I. J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 520-522 (1972).
- Douglas, W. W., and Sorimachi, M., *Br. J. Pharmacol.*, **45**, 143P-144P (1972).
- Orr, T. S. C., Hall, D. E., and Allison, A. C., *Nature*, **236**, 350-351 (1972).
- Butcher, F. R., and Goldman, R. H., *Biochem. biophys. Res. Commun.*, **48**, 23-29 (1972).
- Orci, L., Gabbay, K. H., and Malaisse, W. J., *Science*, **175**, 1128-1130 (1972).
- Colten, H. R., and Gabbay, K. H., *J. clin. Invest.*, **51**, 1927-1931 (1972).
- Davies, P., Fox, R. I., Polyzonis, M., Allison, A. C., and Haswell, A. D., *Lab. Invest.*, **28**, 16-22 (1973).
- Zurier, R. B., Hoffstein, S., and Weissmann, G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 844-848 (1973).
- Day, H. J., and Holmsen, H., *Ser. Haemat.*, **4**, 3-27 (1971).
- Haslam, R. J., in *Physiology of Hemostasis and Thrombosis* (edit. by Johnson, S. A., and Seegers, W. H.), 88-112 (Thomas, Springfield, Illinois, 1967).
- Shepro, D., Belamarich, F. A., Robblee, L., and Chao, F. C., *J. Cell Biol.*, **47**, 544-547 (1970).
- White, J. G., in *Platelet Aggregation* (edit. by Caen, J.), 15-52 (Masson, Paris, 1971).
- Haslam, R. J., *Biochem. J.*, **127**, 34P (1972).
- Haslam, R. J., and McClenaghan, M. D., *Biochem. J.*, **138**, 317-320 (1974).
- Kaliner, M., Orange, R. P., and Austen, K. F., *J. exp. Med.*, **136**, 556-567 (1972).
- Ignarro, L. J., Lint, T. F., and George, W. J., *J. exp. Med.*, **139**, 1395-1414 (1974).
- Estensen, R. D., Hill, H. R., Quie, P. G., Hogan, N., and Goldberg, N. D., *Nature*, **245**, 458-460 (1973).
- Haslam, R. J., and Rosson, G. M., *Am. J. Physiol.*, **223**, 958-967 (1972).
- Mustard, J. F., Perry, D. W., Ardlie, N. G., and Packham, M. A., *Br. J. Haematol.*, **22**, 193-204 (1972).
- Bergmeyer, H.-U., Bernt, E., and Hess, B., *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H.-U.), 736-741 (Academic Press, New York, 1965).
- McPherson, M. A., and Schofield, J. G., *FEBS Lett.*, **24**, 45-48 (1972).
- Butcher, F. R., and Goldman, R. H., *J. Cell Biol.*, **60**, 519-523 (1974).
- Lin, S., Santi, D. V., and Spudich, J. A., *J. biol. Chem.*, **249**, 2268-2274 (1974).
- Spooner, B. S., *Dev. Biol.*, **35**, 113-118 (1973).
- Spudich, J. A., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 585-593 (1972).
- Puskin, E., Puskin, S., Lo, L. W., and Tanenbaum, S. W., *J. biol. Chem.*, **248**, 7754-7761 (1973).

Changed surface glycoprotein as a marker of malignancy in human leukaemic cells

A STRUCTURAL difference in fucose-containing glycopeptides isolated from the surface of virally transformed fibroblasts has been described^{1,2}. Further studies revealed that this difference was caused by increased sialic acid content of the glycopeptides³. Previously we demonstrated⁴ that this phenomenon is not limited to the surface glycoprotein of virally transformed fibroblasts but also occurs in spontaneously chemically or virally transformed cells of fibroblastic, epithelioid and lymphoid morphologies maintained *in vitro*. Since similar results were obtained with mouse lymphosarcoma and rat hepatoma cells grown *in vivo* (ref. 5 and manuscript in preparation), we have extended our observations to human tumour biopsies. We now report that analogous changes occur in the surface glycopeptides of tumour cells obtained from peripheral blood of patients with active leukaemia or with leukaemic transformation of lymphosarcoma. In all cases peripheral blood from untreated patients containing at least 80% leukaemic cells in the white blood cell fraction, were used.

Normal resting and phytohaemagglutinin (PHA) stimulated peripheral blood lymphocytes served as controls. Blood lymphocytes from patients in various stages of infectious mononucleosis (IM) were used as an additional control for a non-malignant lymphoproliferative disease.

Defibrinated blood samples were run on a Ficoll-Isopaque gradient⁶ and lymphocytes or blasts were collected

Table 1 Chromatographic analysis of surface glycopeptides from various leukaemic cells of human origin

Patient	Child/ adult	*Diag- nosis	Recovered WBC mm ⁻³	†Relative elution profiles	
				minus NANase	plus NANase
1	A	ALL	20,000	+++	0
2	C	ALL	5,000	++	nd
3	A	CLL	40,000	+	0
4	A	CLL	27,000	+++	nd
5	A	AML	54,000	+++	0
6	C	AML	120,000	++	0
7	C	CML(ju)	10,000	++	+++
8	A	CML	60,000	+++	+++
9	A	LS	37,000	+	—
10	C	LS	80,000	+	nd
11	A	AML(pro)	3,500	++	—
12	C	AMMoL	107,000	++	0

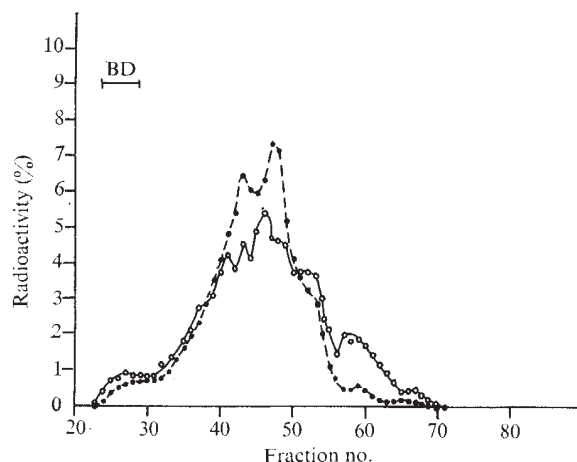
*ALL, acute lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; AML, acute myelocytic leukaemia; CML (ju), chronic myelocytic leukaemia (juvenile type); LS, lymphosarcoma; AML (pro), acute promyelocytic leukaemia; AMMoL, acute myelomonocytic leukaemia.

†The signs denote the number of 0.75 ml fractions that the malignant material eluted ahead (+) or after (—) the control material derived from normal lymphocytes (0, coinciding) as measured after elution of 50% of the radioactivity in the ascending limb of the profile. NANase, neuraminidase treatment; nd, not determined.

from the interphase, washed and counted (Tables 1 and 2: recovered white blood cells (WBC)). Enrichment in myelocytic leukaemic blasts by partial loss of differentiated cells is not excluded by this method. The washed cells were incubated in RPMI 1640 medium supplemented with 20% foetal calf serum and antibiotics in small Erlenmeyer flasks at a density of 4×10^6 cells ml⁻¹. The cells were differentially labelled by incubation for 2 or 3 d in the presence of ¹⁴C-fucose (0.25 μ Ci ml⁻¹) in the case of control lymphocytes or ³H-fucose (0.50 μ Ci ml⁻¹) in the case of tumour cells, IM cells or PHA-stimulated lymphocytes. PHA stimulation was carried out by the inclusion of the mitogen (50 μ g ml⁻¹) in the incubation medium.

Tumour cell numbers did not change during incubation and viability exceeded 90% as measured by trypan blue dye exclusion except in some cases of lymphosarcoma cells (patient 10, Table 1). After incubation, the cells were collected by centrifugation, washed and mildly trypsinised. The solubilised materials from control and tumour, IM or PHA-stimulated cell samples were mixed and exhaustively digested

Fig. 1 Elution profile of L-fucose-containing glycopeptides of pronase-digested trypsinates from differentially labelled PHA-stimulated (●) and resting normal (○) peripheral lymphocytes following filtration through Biogel P10–Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 23–29 and 83–94 respectively.



with Pronase, followed by dialysis and concentration by lyophilisation. The resulting glycopeptides were filtrated over Biogel P10(200–400 mesh)–Sephadex G-50 fine (2:1) columns (1 × 100 cm).

Full experimental details including conditions of neuraminidase treatment of the glycopeptides have been published elsewhere.

Cochromatography of the Pronase-digested, fucose-labelled glycopeptides obtained from resting and PHA-stimulated peripheral lymphocytes showed that the ascending limbs of the elution profiles coincided (Fig. 1). The main peak was increased, but not displaced, in the case of stimulated cells at the expense of lower molecular weight material, similar to the difference observed in log and plateau phase fibroblasts⁷.

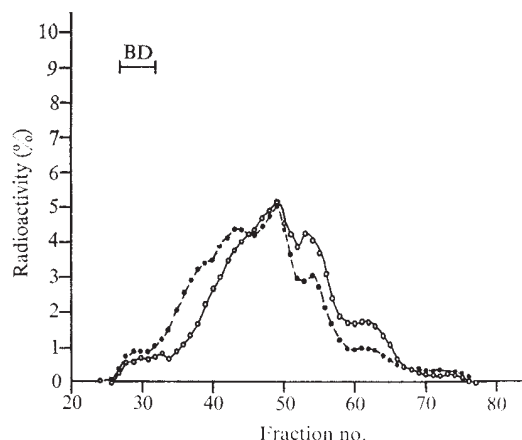


Fig. 2 Elution profile of L-fucose-containing glycopeptides of Pronase-digested trypsinates from differentially labelled peripheral leukaemic cells from patient 4 (Table 1) with CLL (●) and from normal peripheral lymphocytes (○) following filtration through Biogel P10–Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 26–31 and 87–96 respectively.

The results of cochromatography of malignant and normal lymphocyte glycopeptides are summarised in Table 1. In all experiments, including a permanent line of Burkitt's lymphoma (not illustrated), the fucose-labelled glycopeptides of the malignant cells eluted ahead of the controls. The degree of this horizontal displacement was estimated at half-maximal height of the ascending limb of the profiles and expressed as the number of fractions eluting ahead of the control. The difference is represented in Table 1 by the corresponding number of (+) signs. Differences among the elution profiles of individual malignant cell samples were

Table 2 Chromatographic analysis of surface glycopeptides from lymphocytes of patients with infectious mononucleosis

Patient	Child/ adult	Recovered* WBC mm ⁻³	Atypical lympho- cytes (%)	Paul Bunnell* titre	Relative† elution profiles
1	C	16,500	34	1:2048	0
2	A	14,000	33	1:2048	—
3	C	11,000	NR	1:32	—
4	A	5,000	>30	1:1024	0
5	C	5,000	NR	NR	—
6	A	5,000	3	Positive	—
7	C	3,000	36	1:512	0
8	A	2,500	35	1:1024	0
9	C	2,000	>30	1:32	0
10	C	1,500	35	1:512	0

*Normal values: Recovered WBC 1,000–2,000 mm⁻³; Paul Bunnell titre <1:32.

†See Table 1.

||NR, Not reported.

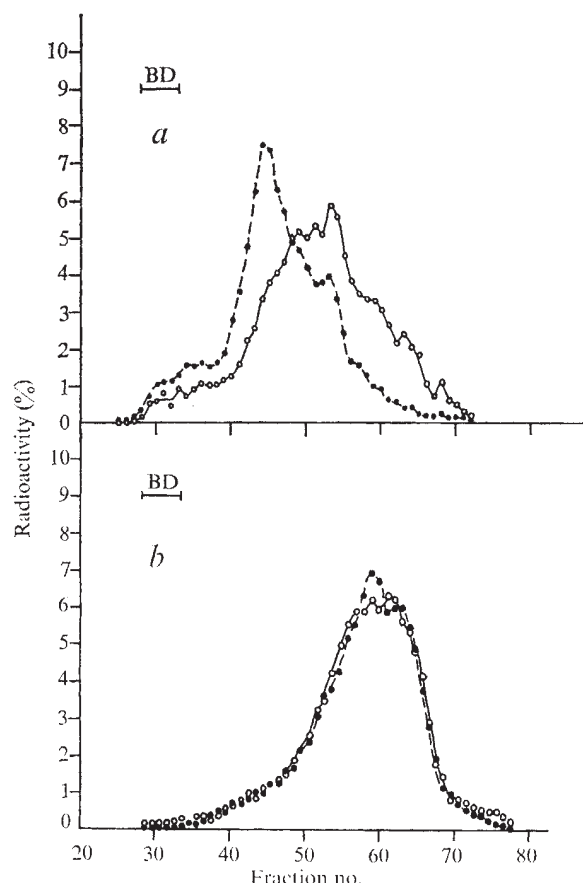


Fig. 3 Elution profiles of L-fucose-containing glycopeptides of Pronase-digested trypsinates from differentially labelled peripheral leukaemic cells from patient 5 (Table 1) with AML (●) and from normal peripheral lymphocytes (○) before (a) and after (b) neuraminidase treatment following filtration through Biogel P10-Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 28–33 and 99–107 respectively.

noted not only in the degree of horizontal displacement but also in the overall shape of the profile, as similarly observed earlier for various transformed cell lines *in vitro*⁴. Thus, the glycopeptides from chronic lymphocytic leukaemia (CLL) cells simply eluted ahead (Fig. 2), whereas AML (acute myelocytic leukaemia) -glycopeptides showed a discrete peak ahead of the main peak of the control (Fig. 3a). It remains to be established whether details of elution profiles correlate with the type of the disease.

The glycopeptides of nine cell samples, each mixed with control glycopeptides, were incubated with *Vibrio cholerae* neuraminidase (Behringer, 50 U ml⁻¹, for 120 min) before cochromatography. In seven cases, the enzyme treatment eliminated the difference in the elution profiles between tumour and normal samples (0, Table 1; Fig. 3b) or caused tumour glycopeptides to elute after the controls by one fraction (—, Table 1). From similar results with cultured normal and transformed cells it has been concluded previously that an increase of neuraminidase-sensitive sialic acid in the transformed-cell glycopeptides is responsible for their being eluted ahead of the corresponding normal material^{3,4}. Apparently the same molecular change occurs in the glycopeptides obtained from the human leukaemic cells studied here.

It may be significant that only in the two cases of chronic myelocytic leukaemia (CML) cells no 'normalisation' of the elution profiles by neuraminidase treatment was obtained. Unpublished results with certain experimental

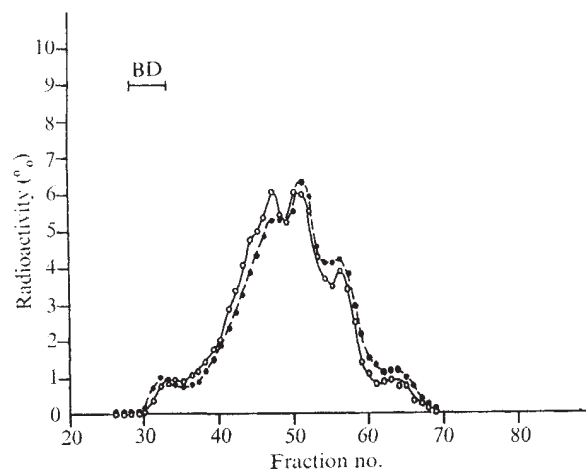
tumours suggest that in such cases either neuraminidase-insensitive sialic acid or an additional anionic (sulphated or phosphorylated) sugar residue could be involved. Further experiments are needed to establish whether CML cells may be distinguished from other leukaemia cases by this criterion and to investigate the determinant of the higher apparent molecular weight of the glycopeptides from these cells.

At the time of the assay, the diagnosis of patient 7 (Table 1) was uncertain. He was suspected of having leukaemia but since the Paul Bunnell test was positive, the possibility of IM could not be excluded. The elution profile of the surface glycopeptides indicated similarity with malignant disease. In the further course of the disease the patient developed the typical clinical picture of CML of the juvenile type.

This result prompted the investigation of lymphocytes from patients with IM. In all 10 cases studied (Table 2), the elution profiles of the glycopeptides coincided with controls of normal lymphocytes or exhibited a small shift to lower molecular weight regions (Fig. 4). Neuraminidase did not materially change the relative elution behaviour of the IM and normal glycopeptides.

The Pronase-digested, fucose-labelled glycopeptides obtained by mild trypsinisation of human leukaemic and lymphosarcoma cells consistently eluted ahead of the corresponding materials from resting or PHA-stimulated lymphocytes. The absence of such difference between resting and PHA-stimulated peripheral lymphocytes (Fig. 1) excludes the possibility that the change in tumour glycopeptides is due to blastoid transformation. The finding that glycopeptides from IM cells eluted together with or slightly after those from normal lymphocytes is further indication that the different behaviour of leukaemic cells is associated with their malignant character. Moreover, it excludes the possibility that the behaviour of the malignant lymphocytes is only the result of the presence of a high count of leukocytes in the peripheral blood since comparable or even higher counts were observed in IM patients. Also, the structural change in surface glycoprotein was consistently found in all malignant cell samples irrespective of their assumed origin as B cells (CLL Burkitt's lymphoma) or as T cells (childhood lymphosarcoma) whereas ALL (acute lymphocytic leukaemia), CML and AML cells lack B and T markers⁸.

Fig. 4 Elution profile of L-fucose-containing glycopeptides of Pronase-digested trypsinates, from differentially labelled peripheral lymphocytes from patient 3 (Table 2) with infectious mononucleosis (●) and from normal peripheral lymphocytes (○) following filtration through Biogel P10-Sephadex G-50 fine (2:1). The radioactivity of the fractions is represented as percentages of total radioactivity eluted from the column. The column markers blue dextran (BD) and phenol red eluted from the column in fraction range 28–33 and 86–94 respectively.



Thus we conclude that surface glycoprotein composition in human leukaemic and lymphosarcoma cells is characteristically changed relative to normal; that except for CML this change is due to an increased content of neuraminidase-sensitive sialic acid; and that the difference is observed in all transformed and malignant cells studied so far, irrespective of the cell type, natural history of the cell, species or oncogenic determinant. This glycoprotein change may be a universal property of tumour cells and related to the expression of the neoplastic phenotype. If not instrumental in neoplastic behaviour, it may at least have diagnostic value.

Finally, we want to point out that the experimental device used here monitors only a minor part of the sialoglycoprotein of the cell surface⁴. Therefore, our observations do not necessarily correlate with other changes observed in the total sialic acid content or its exposition on the cell surface, let alone that in these respects no universal changes have been observed in tumour cells^{9,10}.

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- ¹ Buck, C. A., Glick, M. C., and Warren, L., *Biochemistry*, **9**, 4567-4576 (1970).
- ² Buck, C. A., Glick, M. C., and Warren, L., *Science*, **172**, 169-171 (1971).
- ³ Warren, L., Fuhrer, J. P., and Buck, C. A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1838-1842 (1972).
- ⁴ Van Beek, W. P., Smets, L. A., and Emmelot, P., *Cancer Res.*, **33**, 2913-2922 (1973).
- ⁵ Glick, M. C., Rabinowitz, Z., and Sachs, L., *Biochemistry*, **12**, 4864-4869 (1973).
- ⁶ Boyem, A., *Scand. J. clin. Lab. Invest.*, **21**, 1-109 (1968).
- ⁷ Buck, C. A., Glick, M. C., and Warren, L., *Biochemistry*, **10**, 2176-2180 (1971).
- ⁸ Kaplan, J., Mastrangelo, R., and Peterson, W. D., *Cancer Res.*, **34**, 521-525 (1974).
- ⁹ Weiss, L., in *The Cell Periphery, Metastasis and other Contact Phenomena*, 267-271 (North Holland Publishing Company, Amsterdam, 1967).
- ¹⁰ Emmelot, P., *Eur. J. Cancer*, **9**, 319-333 (1973).

Detoxifying effect of yellow substance on *E. coli* in media containing copper

DISSOLVED yellow organic matter (yellow substance) is ubiquitous in aquatic systems, and is thought to influence the state of ecosystems by affecting the transport and fate of minerals, primary production and, perhaps, the behaviour of aquatic organisms¹⁻⁴. Although much studied, yellow substance remains chemically and physically ill-defined. It is primarily responsible for the yellow colour of particulate-free natural waters which is generally accepted to be the result of the aqueous extract of decaying plant material. One of the primary constituents of plant material, lignin, is the precursor of a class of stable, yellow-brown, intermediate decay products called humins or humic substance⁵. Divided into two fractions, humic acid (base soluble) and fulvic acid (acid soluble), these compounds are insoluble in water and are typically analysed as extracts from soil or sediment. Yellow substance is the remaining yellow decay product and is often called soluble humin (dissolved humic acid). There are data, particularly differences in elemental

composition and visible and ultraviolet light absorption, however, suggesting that yellow substance is a separate compound with cellulose as its source rather than lignin⁶.

There have been two attempts to establish a direct biological role for yellow substance. Prakash and Rashid^{2,3} observed moderate enhancement of growth of various phytoplankton in cultures spiked with yellow substance (referred to as dissolved humic acid in this work); and, in similar experiments, Shapiro¹ reported possible growth enhancement in cultures of several algae. Other findings suggest an interaction with metals in the aquatic ecosystem⁷. Horne and Goldman⁸ reported the suppression of nitrogen fixation in blue-green algae by copper ions (Cu^{2+}). Jones⁹ postulated that metal ions, particularly Cu^{2+} in seawater media suppress *Escherichia coli* in that system. Erickson^{10,11} obtained similar results with *Thalassiosira pseudonana* with the added inference that decomposed natural plankton and detritus, possibly yielding natural chelators¹², reduced Cu^{2+} toxicity. We have now found that although yellow substance plays no direct role in population growth of *E. coli*, it does ameliorate the effects of Cu^{2+} added to the system in toxic concentrations.

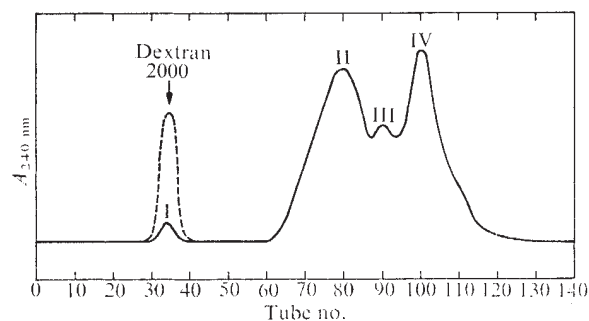


Fig. 1 Chromatogram of parent yellow substance used in this study. Flow rate was 11 ml h^{-1} . Fraction I, recovered at the exclusion limit, was colourless and was not included with parent yellow substance (II-IV).

Yellow substance was obtained from Millipore-filtered ($0.2 \mu\text{m}$) water from the upper Russian River in northern California in October 1973. Isolation was accomplished by ion exchange techniques designed to handle large volumes¹³. Further purification for removal of trace organic material such as free amino acids was accomplished by gel filtration on a 200-cm column of Sephadex G-50 with 0.02 M NaCl as eluant. Resulting chromatograms indicated the possibility of three major fractions (Fig. 1). Comparison of chromatograms of yellow substance obtained on Sephadex G-25 and G-50 with some known standards and with the exclusion limits of the gels indicates an approximate average molecular weight of 5,000. Amicon (UM-02) filtration and lyophilisation yielded an electrolyte-free product which represented approximately 0.001% of the mass of the original filtered water sample.

Cultures of *E. coli* W 1485 (ref. 14) were prepared in a medium containing, (g l^{-1}), K_2HPO_4 , 10.5; KH_2PO_4 , 4.5; MgSO_4 , 1.0; NH_4Cl , 1.0; and glucose, 2.0. The bacteria were cultured aerobically at 37°C with sterile conditions maintained throughout. Cell density was measured by absorbance at 650 nm with a Cary 16 spectrophotometer. For the experiments, all cultures were diluted to an initial optical density of 0.008. After an initial static period, cultures grew exponentially with a mean doubling time of $66.0 \pm 0.9 \text{ min}$ (average and standard deviation of 20 independent cultures). Growth was followed by absorbance measurement every 30 min out to an A_{650} reading of 0.4, representing approximately six generations of reproduction.

To measure the effects of yellow substance on the growth of the population we added it to the culture medium in con-

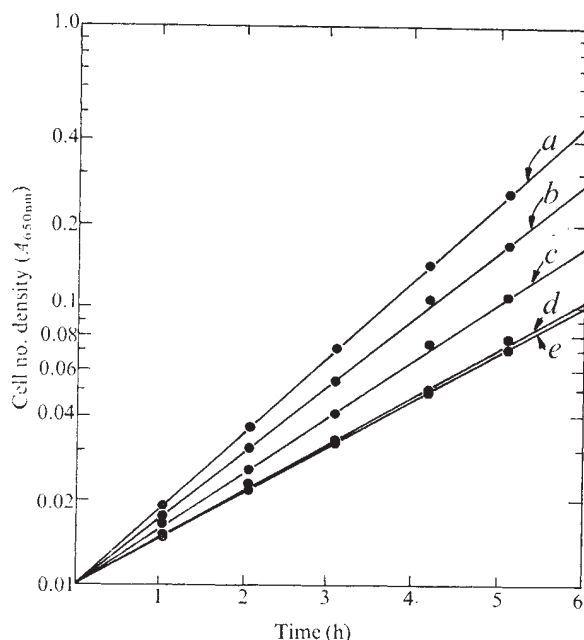


Fig. 2 a, Growth of *E. coli* in control media. b-d, Growth of *E. coli* in media made toxic with 6×10^{-6} M Cu^{2+} with additions of equimolar EDTA (b), yellow substance (c), citrate (d). e, Growth of *E. coli* in toxic control (6×10^{-6} M Cu^{2+}).

centrations of 10^{-6} to 10^{-4} M, assuming an average molecular weight of 5,000 for the yellow substance. Doubling time was not affected by yellow substance. When glucose was replaced by yellow substance, growth stopped.

To investigate the influence of yellow substance on growth in media made toxic with cupric ions, we first established the growth suppressant effect of cupric ions on our *E. coli* system by measuring doubling times in cultures to which CuSO_4 (ultrapure, Johnson, Matthey and Co.) had been added at concentrations of 10^{-4} – 10^{-6} M. There was no growth in the presence of 10^{-4} M Cu^{2+} . At 6×10^{-6} M, growth remained linear but at a substantially reduced doubling time of 108 ± 2.2 min (Fig. 2).

The detoxifying effect of sodium citrate, EDTA and yellow substance was measured in the system made toxic with 6×10^{-6} M Cu^{2+} (Fig. 2). Concentrations of yellow substance of 10^{-5} M significantly enhanced growth rate beyond the toxic control (86 min doubling time compared with 108). Comparison of the doubling time with equimolar EDTA (76 min) and sodium citrate (108 min), indicate that yellow substance is considerably more effective in ameliorating Cu^{2+} toxicity than citrate and comparable with EDTA.

These results suggest that yellow substance should be considered as a prime moderator of metal ion availability to microorganisms in aquatic ecosystems. To ascertain the ultimate influence of yellow substance on ecosystems would require extensive investigation of the effects of the alteration of pH and redox potential on the results we have observed.

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- ¹ Shapiro, J., *Limnol. Oceanogr.*, **2**, 161 (1957).
- ² Prakash, A., Rashid, M. A., Jensen, Arne, and Subba Rao, D. V., *Limnol. Oceanogr.*, **18**, 516 (1973).
- ³ Rashid, M. A., and Prakash, A., *Limnol. Oceanogr.*, **13**, 598 (1968).
- ⁴ Christman, R. F., and Ghassemi, M., *J. Am. Wat. Wks Ass.*, **58**, 723 (1966).
- ⁵ Steelnik, C., *Proc. Calif. Ass. of Chem. Teachers*, **40**, 379 (1963).
- ⁶ Kalle, K., *Oceanogr. mar. Biol. A. Rev.*, **4**, 91 (1966).
- ⁷ Shapiro, J., *J. Am. Wat. Wks Ass.*, **56**, 106Z (1964).
- ⁸ Horne, A. J., and Goldman, C. R., *Science*, **183**, 409 (1974).
- ⁹ Jones, G. E., *Limnol. Oceanogr.*, **12**, 167 (1967).
- ¹⁰ Erickson, S. J., *J. Phycol.*, **8**, 318 (1972).
- ¹¹ Davey, E. W., Morgan, M. J., and Erickson, S. J., *Limnol. Oceanogr.*, **18**, 993–997 (1973).
- ¹² Lewis, A. G., Ramnarine, A., and Evans, M. S., *Mar. Biol.*, **11**, 1–4 (1971).
- ¹³ Milanovich, F. P., thesis, Univ. California, Davis, 1974 (Available as Lawrence Livermore Laboratory Report UCRL-51645).
- ¹⁴ Lederberg, E. M., and Lederberg, J., *Genetics*, **38**, 51 (1953).

A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses

4'-6-DIAMIDINO-2-PHENYLINDOLE (DAPI), which was first synthesised by Dann *et al.*¹ as a trypanocide related to Berenil, has been shown to possess useful DNA binding properties². Thus, DAPI will bind differentially to yeast mitochondrial and nuclear DNA forming highly fluorescent complexes and enhancing the separation of the two DNAs in caesium chloride gradients³. DAPI can also be used as a highly specific fluorescent stain for both nuclear and mitochondrial DNA in yeast. It seems to be highly sensitive and probably permits detection of a single yeast mitochondrial DNA molecule (D.H.W., and D. J. Fennell, unpublished).

We have investigated the use of DAPI in tissue culture systems and have found that it offers a very simple and sensitive procedure for detecting mycoplasma contamination.

Mycoplasmas are a major source of contamination of many cell lines carried in the laboratory, and there are many techniques available for detecting them (for review see ref. 3). Most of these methods are relatively lengthy and involve either cultivation of the mycoplasmas in special broth or agar, rather subjective assessments by cytochemical techniques, or some biochemical measurements often quite complex in nature. Probably the most sensitive and least ambiguous method presently available for the detection of mycoplasmas in tissue culture is that which uses the incorporation of ^3H -thymidine followed by autoradiography of the labelled cells⁴. Discrete and characteristic grains in the cytoplasm and on the cell surface are indicative of the presence of mycoplasmas. This method however, while sensitive and relatively straightforward, does not give the often necessary rapid diagnosis. We have thus developed a procedure for detecting mycoplasmas in tissue culture using DAPI which seems to have all the attributes of the autoradiographic method but is quicker and simpler.

On adding DAPI to tissue culture cells we found that it was rapidly taken up into cellular DNA yielding highly fluorescent nuclei and no detectable cytoplasmic fluorescence under the conditions used (Fig. 1a). If, however, the cells were contaminated with mycoplasmas, characteristic discrete fluorescent foci were readily detected in the cytoplasm and on the surfaces of the cells (Fig. 1b). Similarly, DAPI staining of cells infected with vaccinia virus revealed characteristic fluorescent virus 'factories' in the cytoplasm (Fig. 1c). The method has been applied to a variety of tissue culture cells (HeLa, KB, BHK21, MDBK, LLCMK2) and in all cases where mycoplasmas could be detected by the conventional technique of agar cultivation⁵, cytoplasmic fluorescence was readily observed. In some cases, moreover,

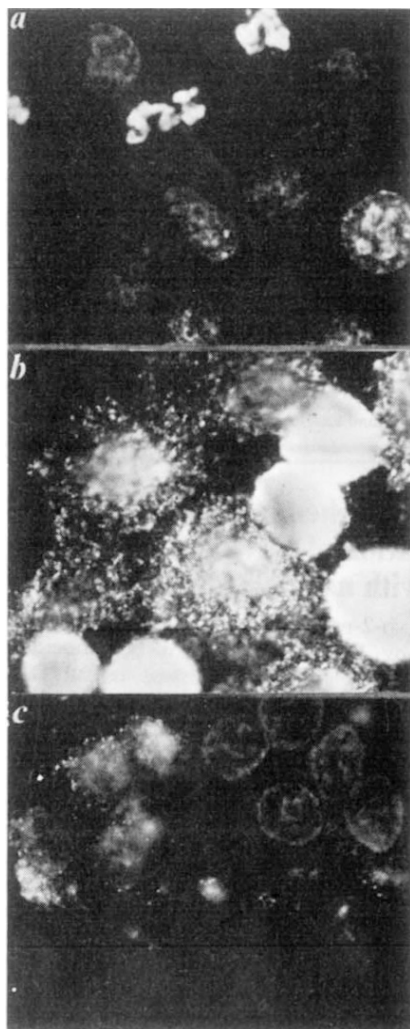


Fig. 1 *a*, Photomicrograph of control, unfixed and uncontaminated HeLa cells, treated with DAPI ($0.1 \mu\text{g ml}^{-1}$) at 37°C for 1 h. Fluorescent nuclei with some mitotic figures can be observed. *b*, Photomicrograph of unfixed, mycoplasma-contaminated HeLa cells treated with DAPI as in *a*. Characteristic cytoplasmic fluorescence can be seen; on refocusing on a different plane, the apparent intense nuclear fluorescence can be seen to be mainly cytoplasmic. *c*, Photomicrograph of unfixed HeLa cells 3 h after infection with vaccinia virus (at an added multiplicity of about 5 PFU per cell). Characteristic 'star-like' fluorescent clusters of presumably maturing virus in 'factories' can be observed ($\times 1,225$).

where there was no clear evidence by agar cultivation, cytoplasmic fluorescence could be observed in a minority of cells, probably indicative of a low level of mycoplasma contamination. A variety of conditions for the incorporation of DAPI into the tissue culture cells were examined, and while it seemed that a fairly wide spectrum of pH, ionic strength and concentration of DAPI could be used, the following simple procedure has been applied on a routine basis for monitoring cells for mycoplasma contamination.

Monolayers of cells grown on coverslips were washed once with phosphate buffered saline (PBS) and incubated in a small volume of PBS containing DAPI at a concentration of $0.1 \mu\text{g ml}^{-1}$ at 37°C for 15–30 min. The coverslip was again washed once with PBS and inverted (without fixation) on a microscope slide and examined in a fluorescence microscope (with a high pressure mercury vapour lamp as a source—for example, Vickers M41 Photoplan—with excitation frequency about 365 nm and emission principally about 450 nm). By lengthening the period of incubation in the presence of DAPI, the intensity of fluorescence in the

nuclei can be increased; with the shorter period of incubation there is less nuclear fluorescence and any cytoplasmic fluorescence is more easily monitored. Background fluorescence is minimal and thus there is no difficulty in discerning fluorescent DNA–DAPI complexes. That the fluorescent foci detected could be attributed to mycoplasmas was tested by deliberately infecting an uncontaminated line of HeLa cells. Mycoplasmas (*Mycoplasma fermentans* PG18) were grown in broth culture and titrated using the indicator techniques as previously described⁵. Monolayers of cells were infected at different added multiplicities and examined at various stages after infection and also after subcultivation of the cells. By using the DAPI technique it was possible both to follow the course of the mycoplasma infection of the cells and also to detect mycoplasmas in the broth cultures. The uninfected cells always gave negative cytoplasmic fluorescence during these procedures while the infected cells showed varying degrees and distribution of the characteristic cytoplasmic fluorescence.

This technique is extremely simple and can be readily applied to monitor tissue culture cells for mycoplasma contamination. Our observations also suggest that the technique may be more sensitive than conventional cultivation techniques and, of course, a cytochemical test obviates the requirements for fastidious culture media. The method should also be useful as a more general tool for monitoring the distribution and properties of DNA in a variety of biological contexts; for example, the course of vaccinia virus infection (Fig. 1c), as the virus matures in the cytoplasm, can be followed readily. We have also successfully used DAPI to follow the fate of inoculum adenovirus at early stages of infection.

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Note added in proof: Since submitting this letter, we have used as an alternative to DAPI, the fluorescent DNA-binding benzimidazole derivative Hoechst 33258 (ref. 6) with broadly similar results.

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¹ Dann, O., Bergen, G., Demant, E., and Volz, G. *Ann. Chem.*, **749**, 68 (1971).

² Williamson, D. H., and Fennell, D. J., in *Methods in Cell Biology* (edit. by Prescott, D.) (Academic, New York, 1974).

³ Kenny, G. E., in *Contamination in tissue culture* (edit. by Fogh J.) (Academic, New York, 1973).

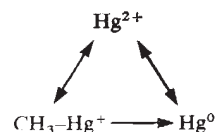
⁴ Nardone, R. M., Todd, J., Gonzalez, P., and Gaffney, E. V., *Science*, **149**, 1100 (1965).

⁵ Russell, W. C., *Nature*, **212**, 1537 (1966).

⁶ Hilwig, I., and Gropp, A., *Expl Cell. Res.*, **75**, 122 (1972).

Biosynthesis and degradation of methylmercury in human faeces

MERCURY is transformed by the microorganisms present in aquatic sediments¹ and rat intestines². Examples of some of the important reactions are illustrated below.



Divalent mercury is reduced to elemental mercury (Hg^0)

aerobically and alkylated to methylmercury (CH_3Hg^+) anaerobically. Methylmercury can be demethylated to Hg^0 and CH_4 aerobically or anaerobically. Methylmercury is of great concern to man because it is highly toxic, is absorbed through the intestinal wall 45 times more rapidly than Hg^{2+} (ref. 3) and is retained in the body longer than Hg^{2+} . The ingestion of food contaminated with methylmercury has resulted in severe outbreaks of mercury poisoning in Minamata, Japan, Alamogordo, New Mexico, and Iran. The ability of bacteria to alkylate mercury prompted us to investigate whether the normal microbial flora of the human intestine could synthesise methylmercury and thus contribute to the body burden of this noxious compound.

Samples of freshly voided human faeces were weighed and placed immediately in anaerobic culture tubes (Bellco glass). Previously reduced culture medium⁴ was added to make a 25% (w/v) suspension of the faecal matter. To avoid killing oxygen sensitive anaerobes, all manipulations were performed in an oxygen-free environment using procedures described by Hungate⁵. The faecal suspensions were gassed with a mixture of H_2 and CO_2 and isolated from air with rubber stoppers. The required amount of $^{203}\text{HgCl}_2$ (New England Nuclear Corp.) or $^{14}\text{CH}_3\text{HgCl}$ (NEN) was added to the gassed mixtures and rapidly mixed. The samples were incubated at 37°C in a shaking water bath. Methane synthesised by the faecal bacteria was measured by gas chromatography on a silicic acid column.

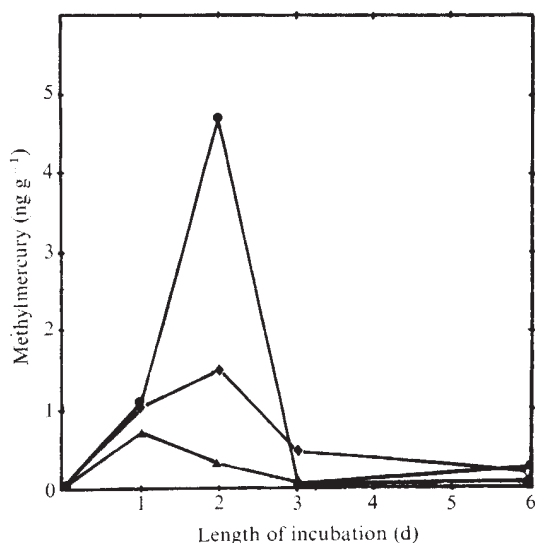


Fig. 1 Concentration of methylmercury in faeces after addition of 5×10^{-7} M HgCl_2 (▲); 1×10^{-6} M HgCl_2 (◆); and 7×10^{-8} M HgCl_2 (●).

Methylmercury was extracted from the faecal suspension by the combined mercuric chloride–cysteine acetate method of Westö⁶. One millilitre of the sample was placed in a centrifuge tube together with 1 ml HgCl_2 (5%, w/v); 1 ml 6N HCl, and 2 ml benzene. The sample was mixed vigorously for 1 min and then centrifuged at $3,000g$ for 5 min at room temperature. The upper (benzene) layer was removed and mixed vigorously with 0.5 ml of 1% cysteine acetate for 1 min. The aqueous layer was removed and mixed thoroughly with 0.2 ml of 6N HCl and 1.0 ml of benzene. The benzene layer was mixed with anhydrous sodium sulphate to remove water.

Methylmercury was identified by thin-layer chromatography of the cyanide and iodide salts. The cyanide salt was made by mixing the benzene layer from the cysteine acetate extraction with 0.5 ml of 1M KCN. The cyanide

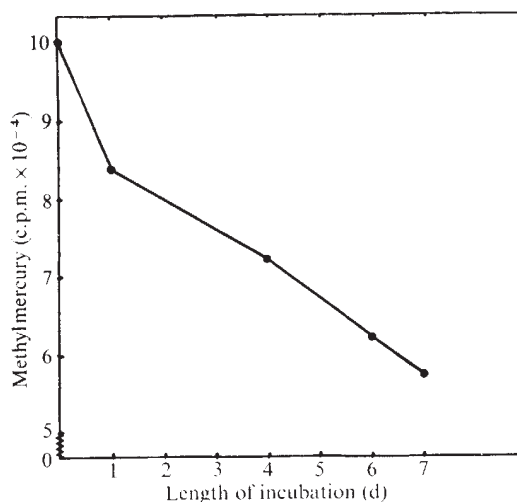


Fig. 2 Disappearance of $^{14}\text{CH}_3\text{Hg}^+$ from faeces. Initial methylmercury concentration was 3.1×10^{-6} M. The $^{14}\text{CH}_3\text{Hg}^+$ was added to anaerobic tubes containing 10 ml of diluted faeces. The tubes were gassed with H_2 : CO_2 (80:20) and incubated at 37°C . One ml samples were withdrawn and extracted for methylmercury as described.

derivative was then chromatographed on silica gel G by thin-layer chromatography using diethyl ether–toluene (90:10, v/v) as the developer. Methylmercury was visualised by spraying with 0.4% (w/v) dithizone in benzene. To verify further that the compound in question was methylmercury, the benzene layer was mixed with NaI and chromatographed using light petroleum–diethyl ether (70:30) as the developer. The presence of ^{14}C -methylmercury or ^{203}Hg -methylmercury (isotopic methylmercury) was established either by scanning the chromatographic sheets in an actigraph (Nuclear-Chicago) or by counting the amount of isotope in silica gel scraped from the sheets.

Methylmercury was produced when faecal matter was incubated anaerobically with $^{203}\text{HgCl}_2$ (Fig. 1). Maximum levels of methylmercury were produced during the first 2 d of incubation followed by a rapid decline. This type of production pattern was repeated in every sample examined, although the peak sometimes appeared as early as 18 h. The amount of methylmercury produced was directly proportional to the amount of Hg^{2+} added. In this experiment the amount of radioisotope was held constant and the amount of non-radioactive Hg^{2+} was varied. No dilution effect was observed, suggesting that a significant proportion of the Hg^{2+} was unavailable for methylation, probably because it was immobilised as the very insoluble sulphide derivative. It is interesting that there was no correlation between methane and methylmercury biosynthesis. Wood *et al.*⁷ reported that cell-free extracts of the methanogenic bacterium *Methanobacterium* strain M.o.H. could catalyse the alkylation of Hg^{2+} . Samples which did not synthesise methane, however, still yielded high levels of methylmercury.

The rapid loss of methylmercury from the samples prompted an investigation into the half-life of the compound in the faeces. These experiments were performed by following the disappearance of a known amount of added $^{14}\text{CH}_3\text{Hg}^+$. As Fig. 2 shows, $^{14}\text{CH}_3\text{Hg}^+$ disappeared at a constant rate during the 7-d test. Thus, either by chemical or biological means, methylmercury can be degraded in an anaerobic faecal specimen. $^{14}\text{CH}_4$ was not observed, thus loss is not the result of reductive demethylation.

These studies are evidence that the microbial flora of the intestine of man has the potential to transform Hg^{2+} to highly toxic methylmercury and could contribute significantly to the methylmercury burden of the body and thereby

add to the risk of incurring or increasing the severity of methylmercury poisoning.

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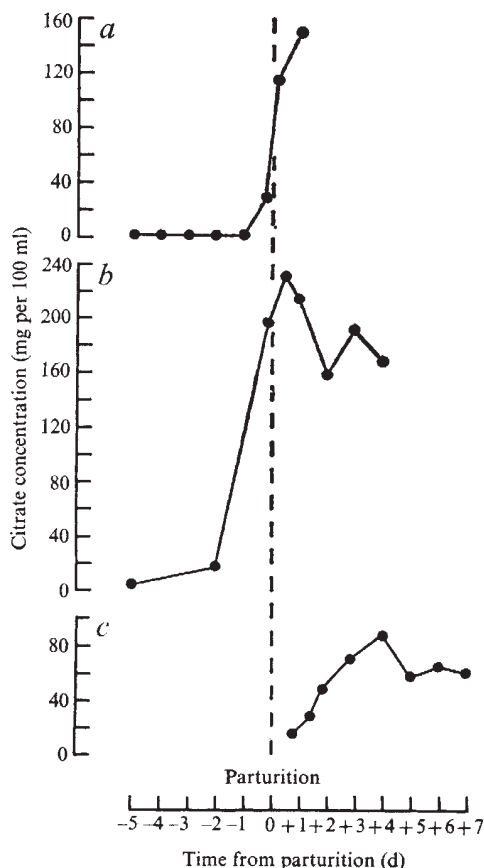
- ¹ Jensen, S., and Jernelöv, A., *Nature*, **223**, 753-754 (1969).
- ² Abdullah, M., Arnesjö, B., and Ihse, I., *Svenska Läkartidningen*, **71**, 810-811 (1974).
- ³ Norseth, T., and Clarkson, T. W., *Archs Environ. Health*, **22**, 568-577 (1968).
- ⁴ Bryant, M. P., and Robinson, I. M., *J. Dairy Sci.*, **44**, 1446-1456 (1961).
- ⁵ Hungate, R. E., *Bact. Rev.*, **14**, 1-49 (1950).
- ⁶ Westöb, G., *Acta Chem. scand.*, **22**, 2277-2280 (1968).
- ⁷ Wood, J. M., Kennedy, F. S., and Rosen, C. G., *Nature*, **220**, 173 (1968).

Citrate in milk: a harbinger of lactogenesis

CONSIDERABLE amounts of citrate (130-160 mg per 100 ml) occur in the milk of cows and goats, and approximately half this amount in that of women. In spite of its central role in the metabolism of all cells, the significance of the presence of citrate in milk and its mode of secretion are not known¹. We report that the final stage of lactogenesis is preceded by the onset of citrate secretion into colostrum.

Matured but previously quiescent epithelial cells suddenly start to secrete large quantities of protein, fat and carbohydrate at about the time of parturition. Although there are significant changes in plasma hormone concentrations in late pregnancy and at term, the nature of this lactogenic trigger remains unknown^{1,2}. Cowie and Tindal³ have suggested that the presence

Fig. 1 Concentrations of citrate in the mammary secretion of *a*, a cow; *b*, a goat; and *c*, a woman about the time of parturition. Small samples were taken from the teat and analysed by the method of White and Davies¹⁰. In all species the young were suckled normally after parturition.



in the secretory tissue of the relevant organelles, enzymes, substrates and some specific milk components, is not sufficient evidence for the onset of copious secretion. For example, udders of cows and goats may contain several litres of a fluid with almost normal milk lactose and ion concentrations 6-7 weeks before term, although copious secretion starts at about the time of parturition (ref. 3 and I. R. Fleet, J. A. Goode, M. H. Hamon, M. S. Laurie, J. L. L., and M. P., unpublished). The onset of secretory activity therefore occurs in two stages, and other investigations^{5,6} have in fact only covered the first stage^{4,5}.

The rate of onset of copious secretion is indeed impressive; in cows the yield may be as high as 40 l d⁻¹ within a few days after parturition and 4 l d⁻¹ in goats. The mammary glands need about 70 g glucose per litre milk formed to support secretion⁶, and although mammary blood flow increases markedly before parturition in goats and cows^{7,8} the uptake of glucose and oxygen does not begin until immediately after parturition and then reaches high levels within a few hours⁸.

In milk from seven goats and seven cows, citrate concentration increased by 3-150 times (median for goats 10 times, for cows 46 times) 1-2 d pre-partum and 2-3 d post-partum (Fig. 1). This rapid increase is illustrated by data from a cow in which the citrate concentration increased from 7 to 110 mg per 100 ml in the last 5 h of pregnancy. In women the onset of copious milk secretion does not begin until 3-4 d post-partum⁹, and it is significant that citrate in samples of secretion obtained from the wife of a colleague on the day of delivery was low and then increased to reach a peak on day 4 (Fig. 1).

Milk citrate is synthesised from glucose and acetate in the mammary gland of the goat⁶. The sudden marked increase in citrate thus indicates not only the onset of synthesis and secretion of this substance but of copious secretion as well.

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- ¹ Linzell, J. L., and Peaker, M., *Physiol. Rev.*, **51**, 564-597 (1971).
- ² Cowie, A. T., and Tindal, J. S., *The Physiology of Lactation* (Arnold, London, 1971).
- ³ Hartmann, P. E., *J. Endocr.*, **59**, 231-247 (1973).
- ⁴ Reynolds, M., and Folley, S. J. (eds), *Lactogenesis* (Univ. Pennsylvania Press, Philadelphia, 1969).
- ⁵ Barry, J. M., *Nature*, **227**, 415 (1970).
- ⁶ Linzell, J. L., in *Lactation* (edit. by Larson, B. L., and Smith, V. R.) **I**, 143-225 (Academic, New York 1974).
- ⁷ Paterson, J. Y. F., and Linzell, J. L., *J. Endocr.*, **62**, 371-343 (1974).
- ⁸ Reynolds, M., in *Lactogenesis* (edit. by Reynolds, M. and Folley, S. J.) 145-151, (Univ. Pennsylvania Press, Philadelphia, 1969).
- ⁹ Hardwick, D. C., Linzell, J. L., and Mepharm, T. B., *Biochem. J.*, **88**, 213-220 (1963).
- ¹⁰ White, J. C. D., and Davies, D. T., *J. Dairy Res.*, **30**, 171-189 (1963).

Isoenzymes of hexokinase in human muscular dystrophy

OF the several genetically determined diseases called muscular dystrophy, the best known, most studied and most severe are the sex-linked Duchenne type and its more slowly progressing variant, the Becker type¹. The aetiology of these dystrophies is unknown, although dystrophies associated with deficiencies of vitamins and other nutrients are known in animals, and myopathy in man may arise from causes as diverse as alcoholism, thyrotoxicosis, infection and autoimmunity². Such variety of known myopathies gave little help to early searches for the biochemical cause of the genetically determined dystrophies³. Recent workers have turned to biochemistry in search of a specific lesion in protein synthesis and its control⁴⁻⁶, or to tissues other than muscle, for example, its motor innervation⁷⁻¹⁰ or vascular supply¹¹. Clearly, a genetic lesion must be translated

into some change in protein synthesis, but this change will have other effects which will probably be more readily detectable. Other tissues besides muscle will be subject to the genetic changes which determine dystrophy, and may also show pathological signs.

Our belief is that dystrophic skeletal muscle will have specific properties from which the primary lesion(s) may be deduced. We have found that in dystrophic muscle, more glucose than usual is converted to fructose instead of to glucose 6-phosphate, under certain metabolic conditions^{12,13}. The conversion of glucose to glucose 6-phosphate is catalysed by hexokinase (EC 2.7.1.1.) which is present in mammalian tissue in one or more of several electrophoretically distinguishable isoenzymes differing considerably in their respective K_m values for glucose¹⁴⁻¹⁶.

Hexokinase activity has previously been measured in extracts of normal and dystrophic human muscle¹⁷⁻¹⁹, and seems to be present in adequate amounts in the test tube at

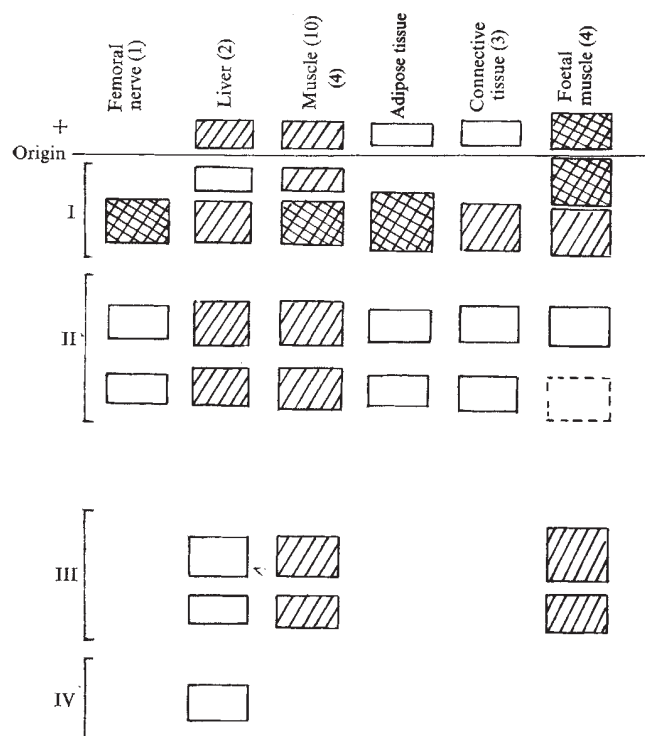


Fig. 1 Hexokinase isoenzymes of normal human tissues. Tissue samples (50–200 mg) were homogenised in 2–20 volumes of 0.1 M Tris, pH 7.4 containing EDTA (5 mM) and glucose (10^{-6} M). Homogenates were centrifuged for 6,000,000g min. Supernatants were used directly for electrophoresis on Cellogel strips (Reeve Angel, London) at 200 V for 1 h in 0.02 M sodium barbital buffer, pH 8.4 containing EDTA (2.7 mM). All these procedures were carried out at 4°C. Staining strips for protein was carried out for 5 min in a solution of 0.2 g Ponceau S and 3 g trichloroacetic acid in 100 ml water. Staining of hexokinase activity took place at three different glucose concentrations for each extract, namely 10^{-2} M, 5×10^{-4} M and 10^{-6} M, according to the method of Katzen, Soderman and Wiley¹⁵. Supernatants were also concentrated tenfold using Minicon polymer blocks (Amicon Ltd, High Wycombe, UK) and again subjected to electrophoresis so that faintly-staining bands should not be overlooked. Bands of hexokinase activity were referred for comparison to the albumin band of the strip stained for protein. The degree of activity is represented by the density of shading. Tissue was used either fresh or after storage at -70°C . Liver and nerve were autopsy specimens. Muscle (four spinalis; six latissimus dorsi) was obtained during orthopaedic operations, and connective tissue and adipose tissue used in this study were trimmed from around such muscle specimens. Foetal muscle was obtained from embryos at therapeutic abortion at 14–18 weeks gestation. Isoenzymes are classified according to Katzen *et al.*¹⁵. The number of specimens used is given in parentheses. There was no detectable difference in the isoenzyme patterns of spinalis and latissimus dorsi muscles.

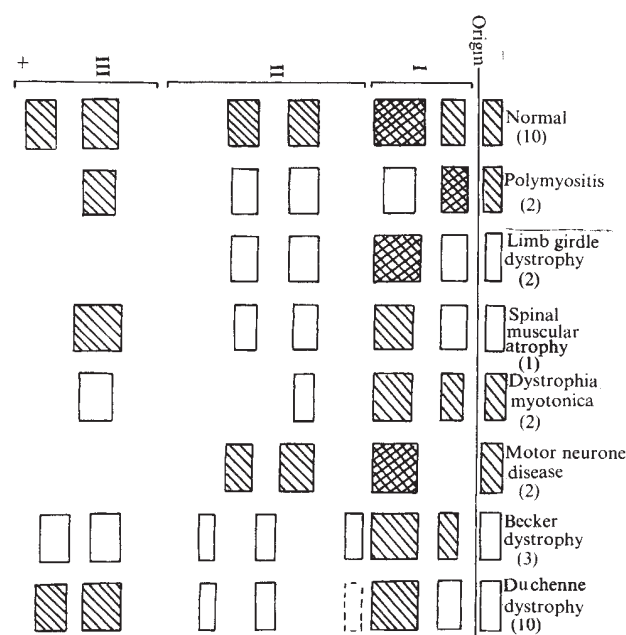


Fig. 2 Hexokinase isoenzymes in human muscle in health and disease. Specimens (10–100 mg) from diagnostic muscle biopsies were all obtained from deltoid, except for four patients with Duchenne dystrophy and two with Becker dystrophy in which the site of biopsy was gastrocnemius. Deltoid muscle was obtained at autopsy from three patients dying at age 18–20 yr from the effects of Duchenne dystrophy. These muscles were grossly affected, and muscle fibres were dissected from the surrounding fibrous and fatty tissue under magnification. The remaining patients with Duchenne dystrophy aged 6–10 yr were still ambulant; microscopic examination of the muscle showed active myopathy. The patients with Becker dystrophy were aged 8–18 yr, all actively ambulant, and showed early myopathic histopathology. Other patients used as controls were of widely differing ages. Treatment of specimens was otherwise as for Fig. 1.

the high glucose concentrations used. We examined the isoenzyme patterns of hexokinase in a variety of tissue samples. Figure 1 shows the normal isoenzymes of human tissues, illustrating the variation in isoenzyme constitution between tissues but the constant electrophoretic mobilities of the bands concerned. Figure 2 demonstrates that the isoenzymes of diseased muscle, while varying in amount, do not differ from the normal isoenzymes in mobility, except in the case of the X-linked dystrophies of Duchenne and Becker. Normally, isoenzyme II consists of two bands on Cellogel, but in X-linked dystrophy there are three, two of which move faster than the bands of normal isoenzyme II, and one more slowly moving band close to isoenzyme I. Isoenzyme II is invariably weakly staining in these X-linked dystrophies, and is sometimes seen only after concentrating the extract. These differences indicate that the protein reacting as isoenzyme II in X-linked dystrophy differs from the normal isoenzyme II. As this isoenzyme is characteristically abundant in skeletal muscle, the change in its properties in dystrophy may be very significant. The alteration in isoenzyme II is not the consequence of adulteration with adipose or connective tissue, nor of reversion to foetal protein type, as these tissues contain only normal isoenzyme II. Furthermore, the change in isoenzyme II has also been shown in three livers, one brain and one sciatic nerve obtained *post mortem* from patients affected with Duchenne dystrophy, and from muscle and liver from a foetus of 18 weeks presumed to have Duchenne dystrophy.

In mixed extracts of normal and dystrophic muscle, the electrophoretic patterns of each constituent tissue were preserved. It was not possible to resolve the normal iso-

enzyme II bands from the slowest and medium bands of dystrophic muscle isoenzyme II but the fastest band of dystrophic muscle isoenzyme II was clearly resolved.

These results suggest that the phenomenon expresses the genetic constitution of the tissues and is not the result of changes consequent upon disease, although it might be a secondary reflection of a yet more general genetic manifestation. Taken in conjunction with our other results^{12,13} it seems more likely that this alteration in hexokinase isoenzyme II, if confirmed, may imply a direct genetic alteration in its catalytic properties, possibly in its substrate affinities, which might restrict glucose flow through the hexokinase step in tissues where isoenzyme II is required.

This would then account for the observed fructose shunt^{12,13} and suggests an aetiology which could embrace the neurogenic⁷, myogenic²⁰ and even the vascular⁸ hypotheses. Nerve damage would be expected to occur from sorbitol formation, as described by Ward, Baker and Davies²¹ in diabetes; muscle would, as suggested earlier^{12,13} suffer from a gradual deposition of triglyceride in and around the fibres; deleterious effects on arterial or capillary function might be envisaged. The involvement of liver as mentioned by Thomson²² would be expected, while the pattern of muscular weakness and degeneration as described macroscopically by Bonsett²³ and microscopically by others^{24,25} would follow if the pathological changes were related to the effects of metabolism of glucose by muscle rather than alternative substrates, such metabolism being consequent on muscular activity²⁶.

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- ¹ Walton, J. N., and Gardner-Medwin, D., in *Disorders of Voluntary Muscle*, third ed. (edit. by Walton, J. N.), 564 (Churchill Livingstone, London 1974).
- ² Gardner-Medwin, D., and Walton, J. N., *ibid.*, 546-560.
- ³ Pennington, R. J. T., *ibid.*, 488-510.
- ⁴ Ionasescu, V., Zellweger, H., and Conway, T. W., *Archs Biochem. Biophys.*, **144**, 51-58 (1971).
- ⁵ Ionasescu, V., Zellweger, H., McCormick, W. F., and Conway, T. W., *Neurology*, **23**, 245-253 (1973).
- ⁶ Kakulas, B. A., and Meyer, W. L., in *Abst. third int. Congr. Muscle Dis.*, Newcastle, No. 228, 98 (Excerpta Medica, 1974).
- ⁷ McComas, A. J., Sica, R. E. P., and Currie, S., *Nature*, **226**, 1263-1264 (1970).
- ⁸ Bradley, W. G., *Nature*, **250**, 285-286 (1974).
- ⁹ Hofmann, W. W., and Thesleff, S., *Eur. J. Pharmacol.*, **20**, 256-260 (1972).
- ¹⁰ Appenzeller, O., Ogini, G., and Palmer, G., in *Abst. third int. Congr. Muscle Dis.*, Newcastle, No. 11, 5 (Excerpta Medica, 1974).
- ¹¹ Hathaway, P. W., King Engel, W., and Zellweger, H., *Archs Neurol.*, **22**, 365-378 (1970).
- ¹² Ellis, D. A., Strickland, J. M., and Eccleston, J. F., *Clin. Sci.*, **44**, 321-334 (1973).
- ¹³ Strickland, J. M., and Ellis, D. A., *Biochem. Soc. Trans.*, **1**, 735-742 (1973).
- ¹⁴ Grossbard, L., and Schimke, R. T., *J. biol. Chem.*, **241**, 3546-3560 (1966).
- ¹⁵ Katzen, H. M., Soderman, D. D., and Wiley, C. E., *J. biol. Chem.*, **245**, 4081-4096 (1970).
- ¹⁶ Katzen, H. M., *Adv. Enzyme Regulation*, **5**, 335-356 (1967).
- ¹⁷ Ronzoni, E., Berg, L., and Landau, W., *Res. Publ. Ass. Res. nerv. ment. Dis.*, **38**, 721-729 (1961).
- ¹⁸ Heyck, H., Laudahn, G., and Luders, C.-J., *Klin. Wschr.*, **41**, 500-509 (1963).
- ¹⁹ Davidenkova, E. F., Verzbinskaya, N. A., Savina, M. V., and Schwartz, E. I., *Zh. Nevropat. Psikiat.*, **70**, 1441-1445 (1970).
- ²⁰ Thomson, W. H. S., Sweetin, J. C., and Elton, R. A., *Nature*, **249**, 151-152 (1974).
- ²¹ Ward, J. D., Baker, R. W. R., and Davies, B. H., *Diabetes*, **21**, 1173-1178 (1972).
- ²² Thomson, W. H. S., *Adv. clin. Chem.*, **7**, 137-197 (1964).
- ²³ Bonsett, C. A., *Neurology*, **13**, 728-738 (1963).
- ²⁴ Dubowitz, V., and Pearce, A. G. E., *J. Path. Bact.*, **81**, 365-378 (1961).
- ²⁵ Baloh, R., and Cancilla, P. A., *Neurology*, **22**, 1243-1252 (1972).
- ²⁶ Narahara, H. T., and Cori, C. F., in *Carbohydrate Metabolism and its Disorders* (edit. by Dickens, F., Randle, P. J., and Whelan, W. J.), **1**, 383 (Academic, London, 1968).

Possible role of prostaglandin F_{2α} in mediating effect of prolactin on RNA synthesis in mammary gland explants of mice

Cyclic nucleotides seem to be intimately involved in the initiation of lactation and the consequent production of milk substances in the mammary gland¹⁻⁵. The ratio of cyclic GMP

to cyclic AMP increased several-fold in mammary glands taken from rats immediately after parturition and during lactation^{1,2}. Further, dibutyl cyclic AMP attenuated or abolished several hormone effects on enzyme activities and other metabolic events in explants from mid-pregnant rats³. It seems that the regulation of the metabolism of the mammary glands of mice may be specifically related to the mechanism whereby prolactin affects this tissue^{4,5}: cyclic GMP mimicked the early action of prolactin on RNA synthesis in mammary gland explants, whereas dibutyl cyclic AMP and agents which inhibit phosphodiesterase abolished this effect. These observations suggest that the early effect of prolactin on RNA synthesis is mediated by an elevated level of cyclic GMP and a reduced or maintained level of cyclic AMP.

Table 1 Effect of various combinations of prolactin, PGF_{2α}, PGE₁, PGA₂ and indomethacin on RNA synthesis in mouse mammary gland explants

Agent added	³ H-uridine incorporation into RNA (d.p.m. per μg RNA)			
	Control	Prolactin	Agent	Agent + prolactin
PGF _{2α}	216 ± 18	395 ± 27	354 ± 18	353 ± 18
PGE ₁	193 ± 16	285 ± 22	182 ± 10	181 ± 19
PGA ₂	198 ± 23	392 ± 28	173 ± 21	171 ± 21
Indomethacin	275 ± 24	462 ± 36	280 ± 32	332 ± 36

Mammary gland explants from mid-pregnant Swiss-Webster mice were preincubated for 2 d in medium 199 containing insulin (2.5 μg ml⁻¹) and hydrocortisone (2.5 μg ml⁻¹) by methods described previously⁷. A PG (50 μg ml⁻¹), indomethacin (10 μg ml⁻¹) and/or prolactin (2.5 μg ml⁻¹) were then added to the explants and incubation was continued for 4 h. ³H-uridine (1 μCi ml⁻¹, 5 Ci mmol⁻¹) was added to the flasks 30 min before terminating the incubations. The specific activity of ³H in the tissue RNA was determined as before⁷.

Numbers are means ± s.e. of explants from seven flasks.

Several investigators have postulated⁶ that the actions of the prostaglandins (PGs) in several biological systems are mediated by cyclic nucleotides. Specifically, it has been postulated that the actions of the PGs of the F series are mediated by cyclic GMP while those of the E and A series are mediated by cyclic AMP. I have therefore tested whether the PGs of the A, E and F series would mimic or attenuate the effect of prolactin on RNA synthesis in mouse mammary gland explants. Table 1 shows that PGF_{2α} mimicked the action of prolactin on RNA synthesis while PGE and PGA₂ abolished the prolactin effect. Moreover, since maximally stimulatory concentrations of prolactin and PGF_{2α} were used, the non-additivity of the two effects is compatible with the possible mediatory role of PGF_{2α} for prolactin. The abolition of the prolactin effect by PGE₁ and PGA₂ may be the result of stimulation of adenyl cyclase⁶ with a consequent enhanced rate of synthesis of cyclic AMP.

Since indomethacin is known to inhibit prostaglandin synthesis through inhibition of the prostaglandin synthetase enzyme, I tested the effect of this drug on the prolactin stimulation of RNA synthesis. Table 1 shows that indomethacin abolished the prolactin effect. These data, therefore, further support the hypothesis that the effect of prolactin on RNA synthesis is mediated by PGF_{2α}. Although an effect of indomethacin unrelated to its effect on prostaglandin synthesis may

Table 2 Time-course for the PGF_{2α} stimulation of RNA synthesis in mouse mammary gland explants.

Incubation time (h)	³ H-uridine incorporation into RNA (d.p.m. per μg RNA)	
	Control	PGF _{2α}
2	280 ± 22	283 ± 13
4	281 ± 16	365 ± 19

Incubation conditions were the same as those described for Table 1 except that the final incubation time was varied. Numbers are means ± s.e. of explants from 14 flasks.

explain the abolition of the prolactin effect, this seems unlikely since indomethacin did not attenuate the effect of $\text{PGF}_{2\alpha}$ on RNA synthesis (data not shown).

Further studies (Table 2) showed that the time-course for the effect of $\text{PGF}_{2\alpha}$ on RNA synthesis did not differ from that reported for prolactin⁷. The effect of $\text{PGF}_{2\alpha}$ on RNA synthesis was apparent after a 4-h incubation but not after a 2-h incubation.

These observations therefore suggest that $\text{PGF}_{2\alpha}$ may be the 'first messenger' for the action of prolactin on RNA synthesis in the mammary gland, whereas cyclic GMP may function as the 'second messenger'. It is interesting that lactation can be initiated in rats⁸ and humans⁹ when parturition is induced by administration of $\text{PGF}_{2\alpha}$. It thus seems likely that after parturition under physiological circumstances, prolactin may contribute to the initiation of lactation by a stimulation of the production of $\text{PGF}_{2\alpha}$.

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- ¹ Sapag-Hagar, M., and Greenbaum, A. L., *Eur. J. Biochem.*, **47**, 303-312 (1974).
- ² Sapag-Hagar, M., and Greenbaum, A. L., *FEBS Lett.*, **46**, 180-183 (1974).
- ³ Sapag-Hagar, M., Greenbaum, A. L., Lewis, D. J., and Hallows, A. C., *Biochem. Biophys. Res. Commun.*, **59**, 261-268 (1974).
- ⁴ Rillema, J. A., *Abstr. Fedn Proc.*, **33**, 214 (1974).
- ⁵ Rillema, J. A., *Hormones metabolic Res.* (in the press).
- ⁶ Kuehl, Jr, F. A., *Prostaglandins*, **5**, 325-340 (1974).
- ⁷ Rillema, J. A., *Endocrinology*, **92**, 1673-1679 (1973).
- ⁸ Deis, R. P., *Nature*, **229**, 568 (1971).
- ⁹ Smith, I. D., Shearman, R. P., and Korda, A. R., *Nature*, **240**, 411-412 (1972).

Endogenous protein kinase activity in nuclear RNP particles from HeLa cells

To elucidate the mechanisms which achieve and regulate the transport from the nucleus to the cytoplasm of those sequences of heterogeneous nuclear RNA (HnRNA) destined to become cytoplasmic mRNA, we have started a systematic investigation of the proteins associated with these RNA species in the form of the so-called 'RNP particles'. The general occurrence of such ribonucleoprotein complexes in eukaryotic cells is widely recognised although they have only recently been described in HeLa cells^{1,2}. The occurrence of phosphorylated proteins in rat brain nuclear particles³, together with the observation that mRNA release from adenovirus-infected KB cell nuclei *in vitro* is an ATP-dependent process⁴, has prompted a search for protein kinase activity in HeLa nuclear RNP particles. The role of protein phosphorylation in the regulation of enzyme activity is now recognised⁵ and we have observed such an activity which does not require the addition of exogenous substrates like histones. The endogenous protein substrates present in HeLa nuclear RNP particles have also been characterised.

RNP particles were spontaneously released from HeLa nuclei during incubation at 37°C (refs 1 and 6) and purified through a sucrose cushion. After incubation in the absence of added protein substrate, these particles are able to catalyse incorporation of radioactive phosphate from $\gamma\text{-}^{32}\text{P}\text{-ATP}$ into acid-insoluble form, the extent of incorporation being proportional to the amount of particles expressed as protein (Fig. 1a). This material was unambiguously identified as phosphoprotein by its insolubility in hot trichloroacetic acid, sensitivity to Pronase and resistance to ribonuclease. Cyclic AMP had no detectable

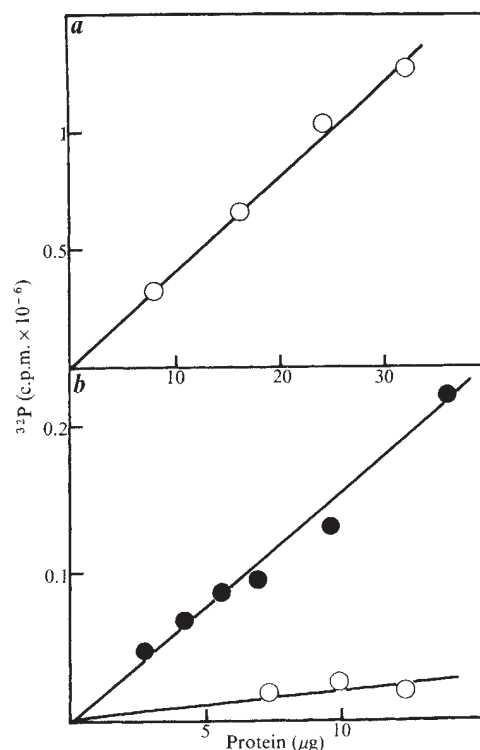


Fig. 1 Protein kinase activity in HeLa nuclear RNP particles. HeLa nuclear particles were prepared from 10⁸ cells essentially as described¹. These 'native particles' were divided into two fractions. One half was kept at 0°C in 0.2 ml TM buffer (0.1 M Tris-HCl (pH 7.8), 10 mM MgCl₂) until use, whereas the other was incubated overnight in TM containing 0.5 M KCl at 0°C. This latter fraction was poured over a 3 ml sucrose cushion (20% w/v) in the same buffer and centrifuged at 55,000 r.p.m. for 4 h at 4°C in the SB405 rotor of an IEC B 60 ultracentrifuge. The pellet of 'washed particles' was resuspended in 0.2 ml TM buffer. The supernatant above the sucrose cushion (0.3 ml) was referred to as 'particles wash'. Protein determination was performed according to Lowry *et al.*⁷ using bovine serum albumin as standard. Reaction mixtures (0.1 ml) containing 2 nmol $\gamma\text{-}^{32}\text{P}\text{-ATP}$ (ref. 8) in TM buffer were incubated at 37°C for 15 min.⁹ The reaction was stopped with 10% trichloroacetic acid, the precipitates collected on glass filters and counted in a Tricarb scintillation spectrometer. *a*, Kinase activity in 'native particles' (○), *b*, kinase activity in 'washed particles' (○) and 'particles wash' (●).

effect on the rate or the extent of the phosphorylation reaction when assayed in the absence of theophyllin. Among the heterogeneous pattern of proteins associated with HnRNA in HeLa nuclei^{1,2}, a major species of molecular weight about 40,000 can be preferentially, although not exclusively, detached from RNP particles by 0.5 M NaCl³. Kinase activity was found to be localised exclusively in the 0.5 M KCl 'particles wash' (Fig. 1b) but not 'washed particles'. As in the case of complete particles, the reaction did not require exogenous substrate, such as histones, indicating that the 'particles wash' contains both the enzyme and its substrate. The absence of detectable activity in washed particles, however, could also reflect the complete removal of the endogenous substrate in addition to that of the enzyme itself. The fact that addition of H1 histone from calf thymus to washed particles did not significantly increase the amount of ³²P incorporated (not shown) renders the first possibility very unlikely unless the enzyme has such a restricted specificity as to be unable to phosphorylate any protein but its own cognate substrates. Although no kinase activity could be detected in the 'washed particles', it is apparent from the comparison of ordinates in Fig. 1a and b that the specific activity of the wash fraction is lower than that of untreated particles. This may result from either a greater enzyme stability and/or a more favourable spatial relationship with the substrates in the 'native particles' than in the soluble 'particles wash'.

We therefore characterised, using SDS-gel electrophoresis, the phosphoproteins produced *in vitro* by native particles (Fig. 2b), and by the 0.5 M KCl wash (Fig. 2d), with reference to the corresponding densitograms of stained gels (Fig. 2a and c). The protein pattern of native nuclear particles (Fig. 2a) is similar to those previously reported^{1,2}, whereas that of the 'particles wash' (Fig. 2c) shows a considerable enrichment in protein species of about 40,000 daltons in agreement with Pederson's report that 'washed particles' are preferentially depleted in this protein species². The distribution of ³²P radioactivity shows two discrete peaks corresponding to molecular weights of about 85,000 and 40,000 for both native particles and 'particles wash'. It has consistently been observed that the 85,000-dalton species is preferentially phosphorylated in the wash fraction while the reverse is true in 'native particles'. A closer association of the kinase with the 40,000-dalton species

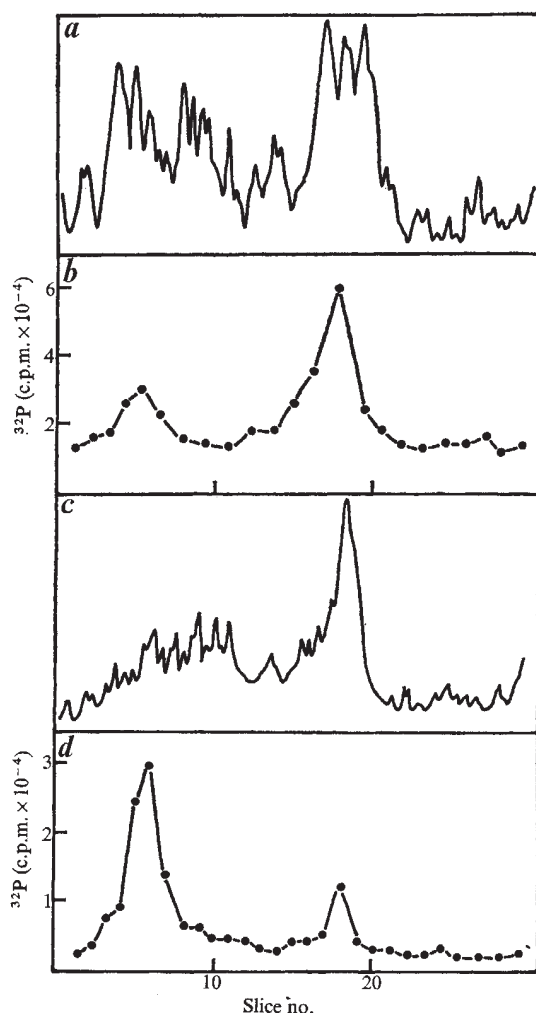


Fig. 2 Sodium dodecyl sulphate-acrylamide gel electrophoresis of proteins from 'native particles' and 'particles wash' incubated *in vitro* with γ -³²P-ATP. Protein from 'native particles' (80 μ g) and 'particles wash' (60 μ g) were incubated in 100 μ l TM buffer in the presence of γ -³²P-ATP as described in the legend to Fig. 1. Denaturation of the samples, electrophoresis on 10% acrylamide gels containing 0.1% sodium dodecyl sulphate, staining of the gels and densitometer tracing were carried out as previously described.¹⁰ Gels were then cut into 1 mm slices and counted directly in a Packard-Tricarb scintillation spectrometer using Cerenkov radiation. Molecular weights were estimated according to Weber and Osborn¹¹, using bovine serum albumin, aldolase and human γ globulin as standards. Densitometer tracings of proteins from 'native particles' (a) and 'particles wash' (c). ³²P radioactivity incorporated into proteins from 'native particles' (b) and 'particles wash' (d).

in 'native particles' can be invoked. An additional phosphorylated species of molecular weight 26,000 has occasionally been observed and this may reflect the occasional presence in some particle preparations of an adventitiously bound protein, possibly a histone, from another nuclear compartment. This point is currently being investigated.

We have described the occurrence of protein kinase activity in the nuclear RNP particles from HeLa cells which can be selectively detached, together with its endogenous substrates, by 0.5 M KCl. A few discrete protein species seem to become phosphorylated in the reaction. While this work was being completed, a similar endogenous protein kinase activity has been reported in rat liver nuclear particles¹² which is able to phosphorylate polypeptides with molecular weights of 25,000, 36,000 and 42,000.

The main proteins released by washing the particles with 0.5 M NaCl have molecular weights of about 40,000 (Fig. 2c and ref. 2). We have recently isolated, using an affinity chromatography on poly(A)-Sepharose column, a phosphorylated protein from HeLa cytoplasm whose size in SDS-acrylamide gels is close to the above value¹⁰. Preliminary experiments indicate that this protein fraction contains kinase activity (unpublished results).

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- ¹ Ducamp, Ch., and Jeanteur, Ph., *Biochimie*, **55**, 1235-1243 (1973).
- ² Pederson, T., *J. molec. Biol.*, **83**, 163-183 (1974).
- ³ Gallinaro-Matringe, H., and Jacob, M., *FEBS Lett.*, **36**, 105-108 (1973).
- ⁴ Raskas, H. J., *Nature new Biol.*, **233**, 134-136 (1971).
- ⁵ Holzer, H., and Duntze, W., *Ann. Rev. Biochem.*, **40**, 345-374 (1971).
- ⁶ Kohler, K., and Arends, S., *Eur. J. Biochem.*, **5**, 500-506 (1968).
- ⁷ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- ⁸ Glynn, I. M., and Chappell, J. B., *Biochem. J.*, **90**, 147-149 (1964).
- ⁹ Dastugue, B., Tichonicky, L., and Kruh, J., *Biochimie*, **55**, 1021-1030 (1973).
- ¹⁰ Blanchard, J. M., Brissac, C., and Jeanteur, Ph., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1882-1886 (1974).
- ¹¹ Weber, K., and Osborn, M., *J. biol. Chem.*, **244**, 4406-4412 (1969).
- ¹² Schweiger, A., and Schmidt, D., *FEBS Lett.*, **41**, 17-19 (1974).

Analysis of X-ray-induced chromosomal translocations in human and marmoset spermatogonial stem cells

ONE of the major classes of genetic damage produced by ionising radiations is heritable reciprocal translocation. In a series of earlier publications, Brewen and Preston¹⁻³ demonstrated that each of several mammalian species had its own unique sensitivity to translocation induction in peripheral leukocytes and that human and marmoset leukocytes, although approximately equal in sensitivity, were twice as sensitive as mouse leukocytes. Brewen and Preston⁴ also reported that in the Chinese hamster and the mouse, the frequency of the translocation type analysed in the peripheral leukocytes was three to four times as high as the frequency of heritable translocations produced in spermatogonial stem cells and recovered in primary spermatocytes. As the mouse is the mammal currently used as the model for estimating man's genetic risk from mutagenic exposure, Brewen and Preston's earlier observations on germ cells should be extended to include a primate and man if possible. Here we present an extension of the interspecific and somatic against germ cell comparisons.

Mature male marmosets were testicularly irradiated with acute doses of 25, 50, 100, 200, or 300 rad of 250 kV X rays. At various intervals after irradiation, depending on the X-ray dose,

bilateral castration was performed, and preparations were made by the method of Evans *et al.*⁵, as modified by Chandley (personal communication). Biopsy material was also obtained from nine men, six of whom had received testicular X-ray exposures of 78, 200, or 600 rad. Details of the irradiation regime are given by Heller and Rowley⁶. The interval between exposure and sampling varied among the individuals, because the programme had been in progress for some time before the cytological investigation was started. Preparations were made by the same procedure as that used for the marmosets. Human and marmoset diplotene-diakinesis figures were analysed for the presence of multivalent configurations (Fig. 1) which were the result of reciprocal translocations induced in spermatogonial stem cells.

The results of this meiotic analysis are presented in Table 1. Previously published data⁷ from mouse studies are also presented in Table 1 for the sake of comparison. Yields of dicentric chromosomes, calculated from the coefficients of regression curves¹, in peripheral leukocytes at the same doses are presented for each species in order that somatic against germ cell comparisons can be made.

The data indicate that, as in the case of the peripheral leukocyte studies, both man and marmoset are at least twice as sensitive to reciprocal translocation induction in spermatogonial stem cells as the mouse. The ratio of somatic cell translocations (that is, dicentrics) to germ cell translocations is approximately 2:1 in man and marmoset at X-ray doses of 100 rad and lower. This variation from the mouse data might be explained by the greater efficacy in observing reciprocal translocations in the primates because the increased chiasmata frequency in primates

Fig. 1 Examples of diplotene-diakinesis figure in marmoset and human primary spermatocytes. *a*, Normal marmoset; *b*, ring-of-IV marmoset; *c*, ring-of-VI marmoset; *d*, normal human; *e*, ring-of-IV human; *f*, ring-of-VI human.

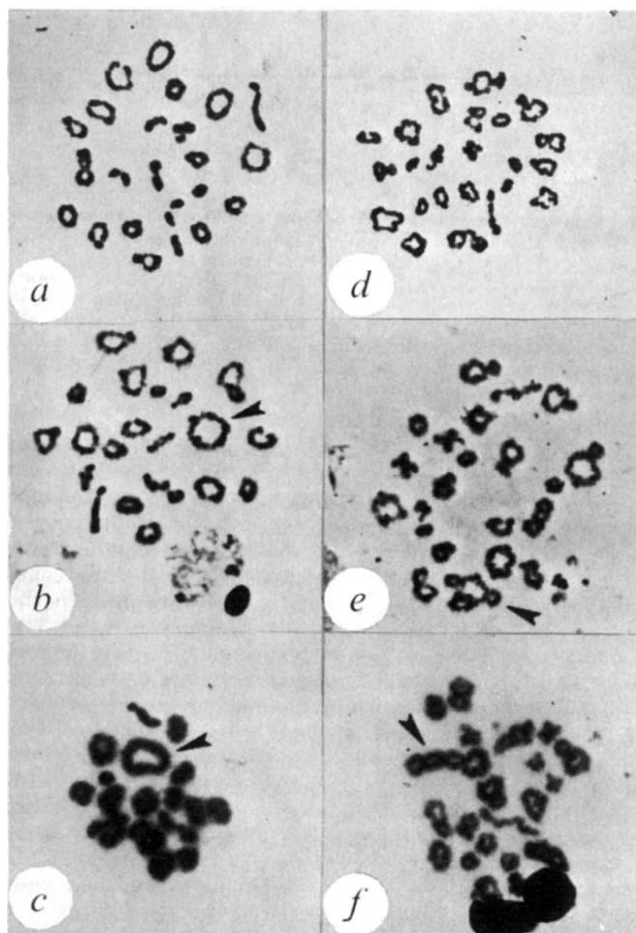


Table 1 Frequencies of reciprocal translocations in meiotic cells and dicentrics in peripheral leukocytes following X-ray exposure to man, marmoset, and mouse

Dose (rad)	Man		Marmoset		Mouse	
	% Dicentrics	% Reciprocal translocations $\pm$ s.e.*	% Dicentrics	% Reciprocal translocations $\pm$ s.e.†	% Dicentrics	% Reciprocal translocations $\pm$ s.e.‡
0	0.0	0.0	0.0	0.0	0.0	0.0
25	—	—	3.0	2.8 ± 0.7	0.9	—
50	—	—	6.7	3.3 ± 0.8	2.1	0.7 ± 0.3
78	8.0	4.0 ± 1.0	—	—	—	—
100	—	—	16.3	7.8 ± 1.1	5.5	1.5 ± 0.5
200	35.2	7.0 ± 1.3	44.6	7.5 ± 1.9	16.4	4.3 ± 0.8
300	—	—	84.9	7.0 ± 1.1	32.7	7.8 ± 1.1
600	250.0	6.1 ± 1.8	—	—	114.0	19.5 ± 1.6

* The number of cells analysed at 78, 200, and 600 rad were 371, 300, and 180, respectively.

† A total of 600 cells were analysed from four testes at each dose, except for 200 rad where 200 from two testes were analysed.

‡ 600 cells were analysed at each dose.

will give a higher probability of a multivalent association being maintained at diplotene-diakinesis. The dose-response characteristics of translocation induction in the germ cells of man and marmoset are similar to those of the mouse. For all three, a peak yield is reached with an apparent decrease at higher doses. This peak yield, however, occurs at approximately 100 rad in the primates whereas it occurs at 500–600 rad in the mouse.

Using the translocation frequencies observed at doses of 100 rad and lower, the mean rate of reciprocal translocation induction in man and marmoset is 7.7×10^{-4} translocations per cell per rad. Using the data of Ford *et al.*¹⁸ on the rate of transmission of cytologically scored translocations to F_1 offspring of irradiated male mice as the lower limit of transmission (that is 1:8), and the expected frequency of transmission of 1:4 as the upper limit, a calculation of transmissible reciprocal translocations per gamete per rad can be made. This value is 0.96×10^{-4} – 1.93×10^{-4} translocations per gamete per rad for acute X-ray exposures.

Jacobs⁹ and Hamerton¹⁰ have summarised the data on the frequency of translocations in live births. The figures are: 0.06% reciprocal translocations, 0.08% Robertsonian translocation, and 0.03% unbalanced translocations. Jacobs *et al.*¹¹ have estimated that the spontaneous rate of viable translocations per gamete per generation is about 4×10^{-4} , of which 2.8×10^{-4} are balanced and 1.2×10^{-4} are unbalanced. This estimate is a minimum as not all translocations can be detected by somatic cell chromosome analysis. So, if it is assumed that the measured spontaneous rate is between 0.25 and 1.00 of the true spontaneous rate, the estimated doubling dose for the induction of reciprocal translocations by acute X rays is 2–16 rad.

In estimating a doubling dose for protracted (that is, chronic) exposures, one precaution must be taken. The experiments to date, using low dose rate irradiation, have been done with large total doses where the dose rate effect is maximised. In making risk estimates the principle concern is for low dose levels where, according to theory and experimental evidence, the dose rate effect for low LET radiations is minimal because of the small contribution by the two-track component. Thus a conservative estimate of a doubling dose for low-dose chronic X-ray exposure might be expected to be the same as for acute low doses with a maximum of 4–30 rad.

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- ¹ Brewen, J. G., Preston, R. J., Jones, K. P., and Gosslee, D. G., *Mutation Res.*, **17**, 245 (1973).
- ² Brewen, J. G., and Preston, R. J., *Environ. Hlth Perspect.*, **6**, 157 (1973).
- ³ Brewen, J. G., and Preston, R. J., *Mutation Res.*, **26**, 297 (1974).
- ⁴ Brewen, J. G., and Preston, R. J., *Nature new Biol.*, **244**, 111 (1973).
- ⁵ Evans, E. P., Breckon, G., and Ford, C. E., *Cytogenetics*, **3**, 289 (1964).
- ⁶ Heller, C. G., and Rowley, M., *Radiat. Res.* (in the press).
- ⁷ Preston, R. J., and Brewen, J. G., *Mutation Res.*, **19**, 215 (1973).
- ⁸ Ford, C. E., Searle, A. G., Evans, E. P., and West, B. J., *Cytogenetics*, **8**, 447 (1969).
- ⁹ Jacobs, P. A., *Proc. 4th int. Cong. Human Genet.*, 232-242 (Paris, 1971).
- ¹⁰ Hamerton, J. L., Ray, M., Abbott, J., and Williamson, C., *Can. med. Ass. J.*, **106**, 776 (1972).
- ¹¹ Jacobs, P. A., Frackiewicz, A., and Law, P., *Ann. Human. Genet.*, **35**, 301 (1972).

Estimation of accessibility of DNA in chromatin from fluorescence measurements of electronic excitation energy transfer

EUKARYOTIC DNA is complexed with proteins which influence its conformation, stability and function^{1,2}. Attempts to correlate the chemical and biological properties of chromatin have focused on the coverage of DNA by chromosomal proteins³⁻⁸. As much as 50% of the DNA in chromatin is accessible to precipitants (such as basic dyes or polylysine)³⁻⁶ or to limiting nucleolytic digestion^{3,4,6-8}, suggesting a model of segmental DNA accessibility in chromatin resembling a frayed telephone wire. Digestion extensively alters chromatin structure, however, and the results of experiments involving precipitation or digestion may be influenced by processes associated with the aggregation phenomena serving as experimental endpoints. We have used an alternative approach to characterise DNA accessibility in chromatin using the transfer of electronic excitation energy between pairs of fluorescent dyes bound to chromatin at low ratios of dye to phosphate. The results suggest that at least one-third of the DNA in chromatin retains a high affinity for basic dyes. The exclusion of dyes or other molecules⁹ from portions of chromatin may, however, depend on the conformation of chromatin as well as on the protein-DNA stoichiometry.

The dyes quinacrine, acridine orange and 33258 Hoechst were chosen as energy donors. All three bind tightly to DNA and chromatin, exhibiting a fluorescence emission spectrum that extensively overlaps the absorption spectrum of the energy acceptor used, ethidium bromide. Following excitation of the donor, energy will be transferred with a high probability to ethidium molecules bound within a critical distance (approximately 25-35 Å) of the donor. Energy transfer efficiency decreases sharply with donor-acceptor separations above this value. The critical donor-acceptor distance for 50% efficient energy transfer, R_0 , determined by individual spectroscopic parameters and relative dye orientations^{10,11}, differs little between DNA and chromatin complexes with any particular donor-acceptor pair, although it varies somewhat between the three donor-acceptor pairs. The relative efficiency of energy transfer between fixed amounts of donor and acceptor bound to either DNA or chromatin depends on the probability that an acceptor molecule will bind within R_0 of the donor and thus, to a first approximation, this energy transfer varies inversely with the acceptor binding space. Hence it provides an estimate of DNA accessibility in chromatin.

Energy transfer in any particular region of chromatin (of length $\gg R_0$) should be proportional to the product of the binding affinities of the donor and acceptor to this segment and will thus reflect the correlation between donor and acceptor binding. Results averaged over many regions will also be weighted according to variations in energy donor fluorescence efficiency. For an accurate estimate of the acceptor binding space, the acceptor should bind to all regions accessible to the donor and the acceptor should not bind preferentially to particular regions of DNA. Quinacrine and ethidium bromide

seem to constitute a suitable donor-acceptor pair, since they both bind to DNA by intercalation in a mutually competitive manner with little base-composition specificity¹²⁻¹⁵.

If ethidium bromide is added to a quinacrine-DNA complex, quinacrine fluorescence is quenched by energy transfer to nearby ethidium molecules, which concomitantly exhibit sensitised fluorescence. At low donor and acceptor saturations, under conditions such that virtually all dye molecules are bound, the donor fluorescence intensity (F) varies inversely with the amount of added acceptor (Fig. 1). The amount of ethidium bromide necessary for a 50% reduction in donor fluorescence (ethidium bromide)₅₀ increases in proportion to the total accessible DNA phosphate concentration (Fig. 2). Quenching of the fluorescence of quinacrine bound to chromatin occurs at about one-third of the amount of added ethidium bromide needed for a comparable effect on quinacrine-DNA complexes (Fig. 2). Direct measurements of quinacrine binding¹⁵ indicate that calf thymus chromatin also possesses about one-third the number of quinacrine binding sites found in an equal amount of DNA, consistent with the existence of an extensive overlap between quinacrine and ethidium bromide binding spaces in chromatin.

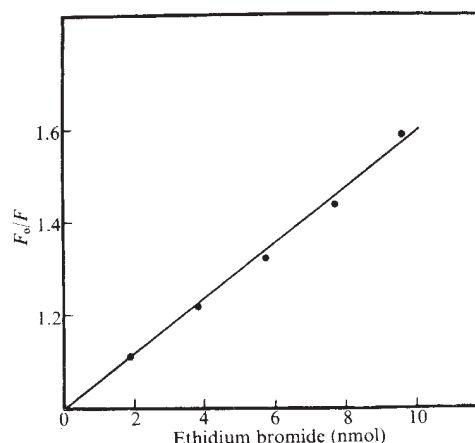


Fig. 1 Energy-transfer quenching of quinacrine fluorescence by ethidium in complexes with calf thymus DNA. The fluorescence of a 2-ml sample of a 0.7×10^{-6} M solution of quinacrine complexed with 2×10^{-4} M calf thymus DNA (in 0.01 M NaCl, 0.005M HEPES, pH 7) was measured after successive 20- μ l additions of a 1×10^{-4} solution of ethidium bromide. Excitation was at 420 nm. Quinacrine fluorescence (F), measured at 493 nm, was corrected for dilution by the ethidium bromide solution and for a very small amount of quenching due to the trivial process of fluorescence and reabsorption. F_0 is the initial fluorescence in the absence of added ethidium. The ratio of F_0/F is plotted against the amount of added ethidium bromide.

Estimates of DNA accessibility in chromatin range from 28%, with acridine orange as the donor to 60%, with the bisbenzimidazole dye 33258 Hoechst (Table 1). This range may reflect variations both in the correlation of donor and acceptor binding distributions and in the quantum yield of donor molecules bound to different DNA sequences. Differences in the mode of binding of donor molecules might also be important. Quinacrine and acridine orange intercalate between DNA bases¹⁴, while 33258 Hoechst, which is a large bisbenzimidazole dye, may occupy one of the grooves of the DNA double helix¹⁷. Preliminary measurements indicate that approximately half of the sites in DNA for 33258 Hoechst binding are available in calf thymus chromatin. If some of these sites are inaccessible to ethidium bromide, the above value of 60% for DNA accessibility in chromatin obtained with 33258 Hoechst as energy donor would be an overestimate. The figures for DNA availability reported here bracket results from experiments involving polylysine or direct measurements of dye binding³⁻⁸. Similar estimates for DNA accessibility in chromatin, using

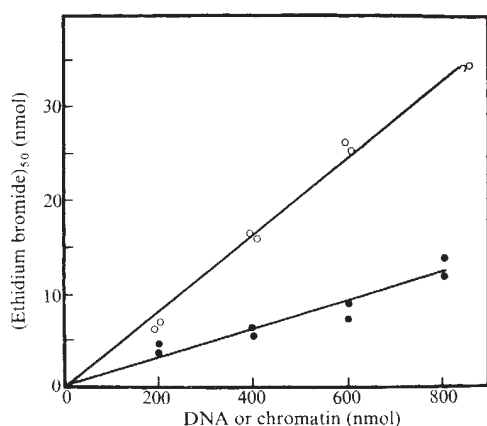


Fig. 2 Energy transfer titration of available phosphate in calf thymus DNA or chromatin. The amount of ethidium bromide at which quinacrine fluorescence is reduced 50%, obtained from data such as those of Fig. 1, is plotted against the amount of calf thymus DNA (○) or calf thymus chromatin (●) present in solution. Data were obtained at ratios of ethidium to phosphate of 0.025 or less. Calf thymus chromatin was prepared by extracting a crude nuclear pellet with 0.024 M EDTA, 0.075 M NaCl, pH 8.0¹⁶, swelling in 0.01 M NaCl, 0.005 M HEPES, pH 7.0, sedimenting through 1.7 M sucrose, and shearing at high speed.

measurements of energy transfer between other dye combinations, have been obtained by Gursky *et al.*¹⁸.

The differences in energy transfer between dye molecules bound to DNA and chromatin can arise if proteins simply exclude dye from some regions without affecting binding in

others¹⁹. They can also occur, however, if donor and acceptor molecules are statistically crowded into limited regions by correlated alterations in binding affinities. To determine whether the higher order structure of chromatin has such an influence on dye binding, energy transfer measurements were made on samples of chromatin either dissolved in 5 M urea or digested briefly with trypsin. Both treatments have been reported to disrupt chromatin structure, as determined principally by circular dichroic and hydrodynamic measurements, with at most a moderate dissociation of protein^{20,21}. The energy transfer between dyes bound to chromatin in 5 M urea was virtually identical to that with DNA (Table 1). Protein dissociation in 5 M urea, estimated by sucrose gradient centrifugation, was negligible. Following trypsin treatment which released less than 30% of the protein from DNA, the apparent accessibility of DNA in the chromatin increased to about three-quarters of that of free DNA (Table 1). Both urea and trypsin treatments increase the apparent accessibility of chromatin to ethidium bromide more than they alter the ratio of protein to DNA. These results are consistent with the idea that reaction of dyes with chromatin depends on chromatin conformation as well as on the literal coverage of chromosomal DNA by protein. It cannot, however, be excluded that a change in the mode of interaction of the residual protein with DNA contributed to our observations.

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Table 1 Estimation of DNA exposure in chromatin using electronic excitation energy transfer

Energy donor	Polymer	(Ethidium bromide) ₅₀ /Pi [†]	DNA accessibility [§]
Quinacrine	DNA	0.044	1.00
	Chromatin	0.016	0.36
	Trypsinised*	0.036	0.83
Acridine orange	Chromatin		
	DNA	0.027	1.00
	Chromatin	0.008	0.28
	Trypsinised*	0.019	0.70
	Chromatin		
33258 Hoechst	DNA in 5 M urea [†]	0.024	1.00
	Chromatin in 5 M urea [†]		
	DNA	0.025	1.03
	DNA	0.041	1.00
	Chromatin	0.025	0.60
	Trypsinised*	0.032	0.78
	Chromatin		
	DNA in 5 M urea [†]	0.039	1.00
	Chromatin in 5 M urea [†]	0.040	1.03

All measurements were made in 0.01 M NaCl, 0.005 M HEPES, pH 7.0, 21–22° C. DNA and chromatin concentrations were between 1×10^{-4} and 4×10^{-4} M. Energy donor concentrations were between 1×10^{-7} and 7×10^{-7} M. 33258 Hoechst (2-(2-(4-hydroxyphenyl)-6-benzimidazolyl)-6-(1-methyl-4-piperazyl)-(benzimidazol-2-yl)-2HCl) was a gift of Dr H. Loewe, Hoechst AG, Frankfurt.

* Chromatin was treated with 8U of tosylphenylchloroketone-treated trypsin (Worthington) per μ mol chromatin DNA phosphate for 2 h at 22° C before measurements were made.

† Urea was added to a final concentration of 5 M in 0.01 M NaCl, 0.005 M HEPES, pH 7.0. Addition of ethidium bromide to DNA plus quinacrine in this solvent did not quench quinacrine fluorescence.

‡ (Ethidium bromide)₅₀/Pi is the estimated ethidium bromide/phosphate saturation of DNA or chromatin at which donor fluorescence is quenched by 50%. In most experiments, this was determined by fitting data like those of Fig. 1 to a straight line after the addition of 8–10 nmol of ethidium bromide. When acridine orange was the energy donor, however, such plots consistently exhibited upward concavity, and initial slopes were estimated using least squares fits of data after the addition of only approximately 5 nmol of ethidium bromide.

§ DNA accessibility is calculated as the ratio of (ethidium bromide)₅₀/Pi in chromatin relative to that with DNA under the same conditions.

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- Simpson, R. T., *Adv. Enzymol.*, **38**, 41–108 (1973).
- Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R. C. C., Marushige, K., and Tuan, D. Y. H., *Science*, **159**, 47–56 (1968).
- Clark, R. J., and Felsenfeld, G., *Nature new Biol.*, **229**, 101–106 (1971).
- Clark, R. J., and Felsenfeld, G., *Nature new Biol.*, **240**, 226–232 (1972).
- Itzhaki, R. M., *Biochem. J.*, **122**, 583–592 (1971).
- Simpson, R. J., and Polacow, F., *Biochem. biophys. Res. Commun.*, **55**, 1078–1084 (1973).
- Itzhaki, R. M., *Biochem. J.*, **125**, 221–224 (1971).
- Clark, R. J., and Felsenfeld, G., *Biochemistry*, **13**, 3622–3628 (1974).
- Simpson, R. T., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2740–2743 (1974).
- Forster, T., in *Modern quantum chemistry*, (edit. by Sinanoglu, O.), 93–137 (Academic, New York, 1965).
- Stryer, L., *Science*, **162**, 526–533 (1968).
- Waring, J. J., *J. molec. Biol.*, **13**, 269–282 (1965).
- Le Pecq, J. P., and Paoletti, C., *J. molec. Biol.*, **27**, 87–106 (1967).
- Lerman, L., *Proc. natn. Acad. Sci. U.S.A.*, **49**, 94–102 (1963).
- Latt, S. A., Brodie, S., and Munroe, S. H., *Chromosoma*, **49**, 17–40 (1974).
- Zubay, G., and Doty, P., *J. molec. Biol.*, **1**, 1–20 (1959).
- Latt, S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3395–3399 (1973).
- Gursky, G. V., Zasedatelev, A. S., and Strelzov, S. A., *Studia Biophys.*, **40**, 105–108 (1973).
- Angerer, L. M., Georgiades, S., and Moudrianakis, E. N., *Biochemistry*, **13**, 1075–1082 (1974).
- Bartley, J. A., and Chalkley, R. J., *Biol. Chem.*, **247**, 3647–3655 (1972).
- Simpson, R. T., *Biochemistry*, **11**, 2003–2008 (1972).

Concanavalin A cap formation on polymorphonuclear leukocytes of normal and beige (Chediak-Higashi) mice

TREATMENT of a variety of cell types with concanavalin A (con A) leads to the aggregation of the lectin into one region or pole of the cell, forming a so-called cap. Not all cells cap (erythrocytes and normal cultured fibroblasts do not). Cells which do cap can be distinguished as (a) lymphocytes¹ and rabbit exudate polymorphonuclear leukocytes (PMN) (our data) that cap directly when incubated with low concentrations of con A at 37° C, and (b) cells exemplified by SV40-transformed fibroblasts² and lymphocytes incubated with high (more than $5 \mu\text{g ml}^{-1}$) concentrations of con A¹ that cap only after incubation with colchicine which

Table 1 Distribution of FITC-con A on PMN from normal and beige (CH) mice

Line	Preincubation	Postincubation	Normal (%)			CH (%)		
			Random	Capped	Patchy	Random	Capped	Patchy
1	Buffer	None	62	11	27	33	44	23
2	Colchicine (10^{-6} M)	None	23	55	22	32	50	19
3	Lumicolchicine (10^{-6} M)	None	73	11	16	—	—	—
4	Cyclic GMP (0.5×10^{-4} M)	None	70	8	22	76	10	14
5	Carbachol (10^{-5} M)	None	87	2	11	72	8	20
6	PMA (10 ng ml^{-1})	None	77	6	17	69	6	25
7	Guanine (0.5×10^{-4} M)	None	—	—	—	25	46	29
8	Guanosine (0.5×10^{-4} M)	None	—	—	—	30	46	24
9	GMP (0.5×10^{-4} M)	None	71	9	20	35	47	18
10	Cyclic AMP (10^{-8} M) + theophylline (10^{-3} M)	None	67	8	25	41	38	24
11	Cyclic GMP (0.5×10^{-4} M) + colchicine (10^{-6} M)	None	72	12	16	73	5	22
12	Carbachol (10^{-5} M) + colchicine (10^{-6} M)	None	76	10	14	—	—	—
13	PMA (10 ng ml^{-1}) + colchicine (10^{-6} M)	None	75	14	11	—	—	—
14	Buffer	Buffer	70	10	20	—	—	—
15	Buffer	Colchicine (10^{-6} M)	43	38	19	—	—	—
16	Buffer	Cyclic GMP (0.5×10^{-4} M) + colchicine (10^{-6} M)	65	15	20	—	—	—
17	Cyclic GMP (0.5×10^{-4} M)	Colchicine (10^{-6} M)	45	33	22	—	—	—

Cell monolayers were preincubated at 37°C in modified Hanks medium containing 5 mM glucose plus the drugs indicated. After 30 min, fresh glucose-free medium containing FITC-con A ($5 \mu\text{g ml}^{-1}$) plus the same concentration of drugs was added and incubation was continued for 15 min. Cells were rinsed and either fixed immediately or postincubated for 20 min at 37°C with medium containing drugs before fixation. Results were obtained by examining 100 cells at random and assigning each cell to one of the 3 labelling categories (see text) according to the distribution of fluorescence. All experiments were done using blood from one normal and one CH mouse (3–5 weeks old) and with the same solutions of drugs and FITC-con A. The data in lines 1–13 are results from one representative experiment and those in lines 14–17 from a separate experiment. Some variation was observed between blood obtained from different animals, but qualitatively all effects were the same. The range of capping in the absence of drugs varied between 8 and 19% in 11 normal mice and between 36 and 58% in 11 CH mice. In all experiments, about 20% of cells showed a patchy labelling with FITC-con A.

disrupts microtubules (MT). These differences between cell types are not understood.

We report here that peripheral blood PMN from normal mice (C57/6J $+/+$ and $+/bg$) do not cap with con A except after colchicine treatment. This colchicine-induced capping is antagonised by cyclic GMP and agents that stimulate its production. In addition, we show that PMN from beige or Chediak-Higashi (CH) mice (C57/6J bg/bg) cap spontaneously with con A and that capping on these cells is also blocked by cyclic GMP. These results suggest a role for cyclic GMP in the regulation of surface phenomena that depend on MT and form the basis for a new hypothesis to explain the pathogenesis of the CH syndrome.

Mouse peripheral blood PMN were obtained as before³ and suspended in modified Hanks medium containing 5 mM glucose. Fluorescein-conjugated con A was prepared by incubating fluorescein isothiocyanate (FITC, Sigma) at a concentration of 0.05 mg mg^{-1} con A for 1 h at 0°C in the presence of 100 mM glucose (to protect the binding site) and phosphate buffer, pH 7.5, and purifying the product after dialysis by affinity chromatography on Sephadex G-50². Monolayers of cells were allowed to form on glass coverslips⁴ and labelled at 37°C with $5 \mu\text{g ml}^{-1}$ con A for 15 min, then rinsed in modified Hanks solution, fixed in 2% paraformaldehyde at 37°C for 10 min and wet mounted. The labelled cells were observed by combined phase-fluorescence using a Zeiss epi illuminator with an FITC interference filter, 450 dichroic mirror, 53 barrier filter, and a 100 neofluar phase objective.

The observed fluorescence was categorised according to three labelling patterns: (1) random or diffuse; (2) cap—either a polar shell of fluorescence or a more concentrated knob or protrusion; (3) patchy—clumped aggregates—often perinuclear, or occasionally peripheral, or as larger more central irregular clusters. Examples of each labelling pattern can be seen in Fig. 1. Treatment of labelled cells with 50 mM α -methyl mannoside removed labelling of the first two patterns (diffuse or cap). Aggregates were not removed, however, indicating that the third or patchy pattern corresponds to endocytosed con A that is inaccessible to the

sugar. This endocytosed con A shows a superficial resemblance to surface (centrally located) caps. The development of the observed patterns seems to follow the sequence 1 to 2 to 3, or 1 to 3 directly (that is, endocytosis without an intermediate cap stage).

Table 1 is a summary of the patterns of fluorescence distribution observed with peripheral blood PMN from normal and CH mice. There was a striking increase in the proportion of capped cells and a corresponding decrease in the proportion of cells showing a random distribution of fluorescence in CH-PMN as compared with PMN from homozygous ($+/+$) or heterozygous ($+/bg$) normal mice (line 1). This difference is illustrated in Fig. 1a and b. Preincubation of monolayers for 30 min with colchicine promoted cap formation in normal PMN but had little additional effect on the extreme degree of capping in CH-PMN (lines 1 and 2). After colchicine treatment the degree of capping in normal PMN was very similar to that in untreated CH-PMN (compare Fig. 1b with c). Lumicolchicine (line 3), a photochemical derivative of colchicine that does not affect microtubules, did not affect capping.

The effects of cyclic nucleotides on capping were investigated because these compounds have been implicated in regulation of colchicine-sensitive (microtubular) functions in several systems. Thus both 3',5'-cyclic adenosine monophosphate (cyclic AMP) and compounds which increase its intracellular concentration, and colchicine, reduce antigen-induced histamine release⁵ and endocytosis-induced release of lysosomal enzymes⁶ from leukocytes. In contrast, both cyclic GMP and agents that elevate its intracellular concentration and deuterium oxide which favours MT assembly, augment histamine and enzyme release in the same *in vitro* models.

Our data show that preincubation of cell monolayers for 30 min with cyclic GMP (line 4) as well as carbamylcholine (carbachol; line 5) and phorbol myristate acetate (PMA, line 6) at concentrations which elevate cyclic GMP levels in PMN^{6,7} caused a marked reduction in capping on CH-PMN and slightly reduced capping on normal PMN. In fact, the degree of capping on CH-PMN after addition of

cyclic GMP was virtually identical to the untreated normal PMN (compare Fig. 1a with d). This effect was chemically specific for the cyclic guanine nucleotide since the potential products of its degradation, guanine (line 7), guanosine (line 8) and 5'-GMP (line 9) did not affect capping. In addition, cyclic AMP (line 10) had no significant effect on cap formation on either CH or normal cells.

When normal cells were preincubated with cyclic GMP (line 11), carbachol (line 12) or PMA (line 13) simultaneously with colchicine, the characteristic stimulation of capping by colchicine (compare line 2) did not occur (Fig. 1e and f). Thus cyclic GMP and compounds which increase intracellular concentrations of this nucleotide antagonised the extensive capping of con A that occurred on both untreated CH-PMN and colchicine-treated normal PMN. The effect of cyclic GMP was readily reversible, indicating that the observed effects occurred without loss of cell viability. Cap formation on normal PMN was enhanced by incubation with colchicine both before (line 2) and after (line 15) labelling with FITC-con A and was antagonised by the simultaneous presence of cyclic GMP in both cases (lines 11 and 16). Cells incubated with cyclic GMP before labelling and with colchicine after labelling (line 17), however, showed a similar increase in con A capping to cells exposed to colchicine alone.

Two mechanisms have been suggested to explain the effect of colchicine on cap formation. According to one hypothesis¹, high concentrations of con A induce an association between intracellular colchicine-binding proteins (presumably MT) and the cell surface that inhibits the lateral migration of bound ligands within the membrane, and colchicine, by virtue of its effects on MT, permits

this movement of surface constituents to resume. A second hypothesis^{8,9} is that cell or membrane movements are required to aggregate lectin-receptor complexes: con A immobilises the cell and colchicine promotes capping only under conditions in which it can restore cell movement. We have shown that cyclic GMP, which stimulates both random and directed (chemotactic) cell movement⁷ does not enhance capping by con A on normal PMN. Further, cyclic GMP antagonises the increase in capping that is induced by colchicine in normal PMN. These observations are consistent with the first hypothesis, in which capping is independent of cell movement.

We have also shown increased capping by con A on PMN from CH mouse. This mutant^{10,11}, like mutants of mink¹² and cattle¹³, is a homologue of the Chediak-Higashi syndrome of man¹⁴, a rare autosomal recessive disorder characterised by partial albinism, the presence of giant granules in most granule-containing cells, impaired leukocyte chemotaxis and defective lysosomal degranulation. The current view of the CH syndrome is that subcellular granule membranes may be defective in structure or function^{15,16}. The apparent increase in the mobility of lectin-receptor complexes on the plasma membranes of PMN from CH mice, which results in extensive cap formation, indicates that this view should now be extended to include other membranes. Furthermore, membranes of CH-PMN behave like membranes of colchicine-treated normal cells in which MT are depolymerised, in terms of their capping response to con A. This could show that the fundamental defect may be an abnormality in MT polymerisation and/or in the interaction between membranes and MT.

The intracellular mechanisms that regulate MT assembly are not yet understood. Our observation, however, that cyclic GMP antagonises the increased con A capping due to colchicine in normal PMN suggests that the cyclic nucleotide may promote MT polymerisation. Reports that cyclic GMP levels increase in lymphocytes¹⁷ and human PMN (J.M.O. and R.B.Z., unpublished) after binding of con A and that the number of MT seen by electron microscopy in human PMN is increased by agents that elevate cyclic GMP¹⁸ are consistent with this proposal. We suggest that in normal PMN binding of con A may elevate cyclic GMP and thereby promote MT assembly. In CH-PMN the generation of cyclic GMP in response to con A may be inappropriately low.

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- Edelman, G. M., Yahara, I., and Wang, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1442 (1973).
- Rozenblith, J. Z., Ukena, T. E., Yin, H. H., Berlin, R. D., and Karnovsky, M. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1625 (1973).
- Weissmann, G., Dukor, P., and Zurier, R. B., *Nature new Biol.*, **231**, 131 (1971).
- Hawkins, R. A., and Berlin, R. D., *Biochim. biophys. Acta*, **173**, 324 (1969).
- Bourne, H. R., Melmon, K. L., and Lichtenstein, L. M., *Science*, **173**, 743 (1971).
- Zurier, R. B., Weissman, G., Hoffstein, S., Kammerman, S., and Tai, H. H., *J. clin. Invest.*, **53**, 297 (1974).
- Estensen, R. D., Hill, H. R., Quie, P. G., Hogan, N., and Goldberg, N. D., *Nature*, **245**, 458 (1973).
- Unanue, E. R., and Karnovsky, M. J., *J. exp. Med.* (in the press).
- Edidin, M., and Weiss, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2456 (1972).
- Gallin, J. I., Bujak, J. S., Patten, E., and Wolff, S. M., *Blood*, **43**, 201 (1974).
- Bennett, J. M., Blume, R. S., and Wolff, S. M., *J. Lab. clin. Med.*, **73**, 235 (1969).
- Lutzner, L. A., Tierney, J. H., and Benditt, E. P., *Lab. Invest.*, **14**, 2063 (1966).
- Padgett, G. A., Leader, R. W., Gorham, J. R., and O'Mary, C. C., *Genetics*, **49**, 505 (1964).
- Blume, R. S., and Wolff, S. M., *Medicine*, **51**, 247 (1972).
- Wolff, S. M., Dale, D. C., Clark, R. A., Root, R. K., and Kimball, H. R., *Anals int. Med.*, **76**, 293 (1972).
- White, J. G., *Blood*, **28**, 143 (1966).
- Hadden, J. W., Hadden, E. M., Haddox, M. K., and Goldberg, N. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3024 (1972).
- Goldstein, I. M., Hoffstein, S. T., and Weissmann, G., *Ann. N.Y. Acad. Sci.* (in the press).

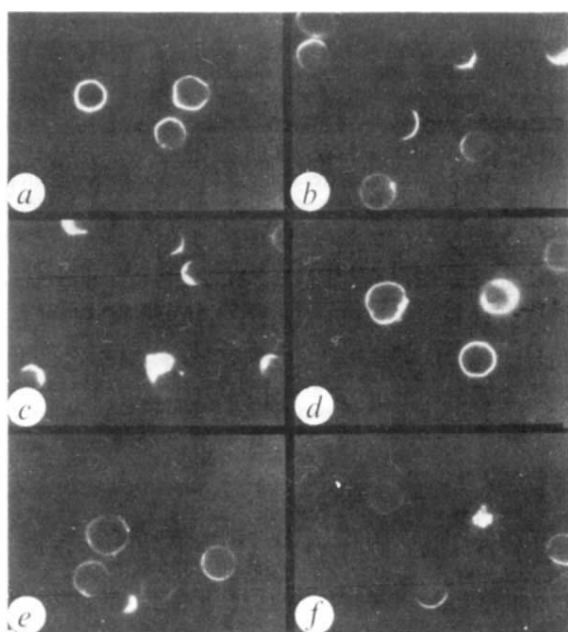


Fig. 1 The distribution of FITC-con A on peripheral blood PMN from normal and beige (CH) mice. *a*, Normal PMN: the diffuse labelling pattern predominates. *b*, CH-PMN: most cells are capped. *c*, Normal PMN incubated with $1 \mu\text{M}$ colchicine: the degree of capping is similar to CH-PMN without colchicine. In one cell the cap has begun to be internalised and fluorescence is seen in a clumpy aggregate. *d*, CH-PMN incubated with $0.5 \times 10^{-4} \text{M}$ cyclic GMP: the labelling pattern is diffuse as in the normal PMN. *e*, Normal PMN incubated with both cyclic GMP and colchicine: cyclic GMP antagonises the increased cap formation seen with colchicine alone. *f*, CH-PMN incubated with both cyclic GMP and colchicine: most of the label is distributed randomly. One cell shows a central cluster of fluorescence that resembles a central cap. This fluorescence cannot, however, be removed by α -methylmannoside and so it most likely represents internalised con A.

Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules

PARASITIC helminths characteristically provoke high levels of IgE antibodies in man and similar reagin-type antibodies in animals. But it has not been demonstrated conclusively that these antibodies have a protective role.

When they are incubated in the serum of Fisher rats immune to *Schistosoma mansoni*, the peritoneal adherent cells of normal inbred Fisher rats are strongly adherent to *S. mansoni* schistosomules maintained *in vitro*^{1,2}. Electron microscopy has shown that the adherent cells are macrophages. Here we report experiments which indicate that the factors in immune serum responsible for the macrophage adhesion are specific anti-schistosome IgE antibodies.

Serum samples were obtained by bleeding infected Fisher rats at various intervals after exposure to 400 cercariae of *S. mansoni* (group 1). Uninfected Fisher rats were used as a control (group 2). A highly significant correlation was obtained between the percentage of macrophage-coated schistosomules and the time elapsed since infection ($r=0.562$; $n=80$; $P<0.001$).

After infection for 30 d, the mean percentage of macrophage-coated schistosomules was significantly higher in group 1 than in group 2 (Fig. 1). After 50 d, the mean percentage of macrophage-coated schistosomules was 88.4% (s.d. 9.4; $n=8$) compared with a value for the uninfected control sera of 29.8% (s.d. 23.1; $n=6$; $P<0.001$). The control values could be reduced to a mean of 10% if sera from uninfected germ-free rats were used (A.C., J.-P.D., M.C., and H.B., unpublished).

Adherence of immune serum-incubated macrophages was inhibited when the serum was heated at 56° C for 3 h and

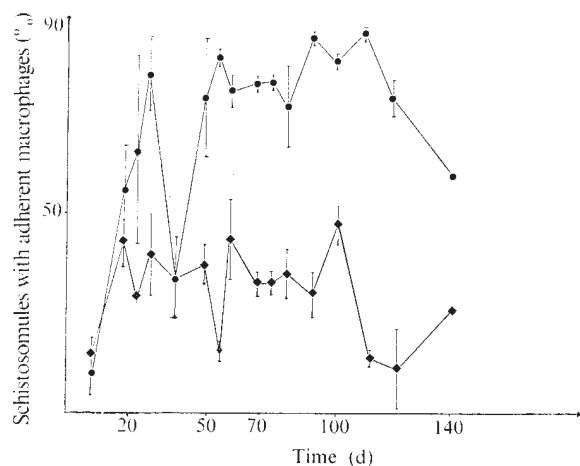


Fig. 1 Relationship between the adherence of serum-incubated macrophages and the period of infection of rats by *S. mansoni* cercariae. Normal syngenic Fisher rat macrophages were collected by peritoneal washing with Eagle's MEM and plated in Falcon dishes. Non-adherent cells were washed off after 3 h incubation. Adherent population was incubated in MEM containing 20% of either immune (group 1) or normal (group 2) rat serum. At least five replicates in each group were performed. Approximately 200 *S. mansoni* schistosomules recovered according to the technique described by Clegg and Smithers³, were added and adherence of macrophages was estimated after 3 h contact under an inverted phase contrast microscope. More than 30 adherent macrophages to one schistosomule was considered to be significant and the percentage of embedded schistosomules was calculated. ●, Group 1; ◆, group 2; s.d. are indicated by vertical lines through points. Statistical analysis (Student's *t* test and Wilcoxon two samples test) showed highly significant ($P\leq 0.01$) differences between the two groups.

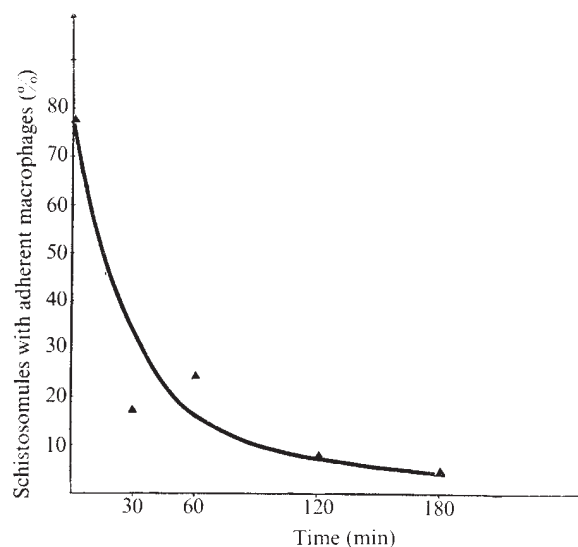


Fig. 2 Thermolability of serum factor involved in macrophage adherence to *S. mansoni* schistosomules, as estimated by heating infected rat serum 30–180 min at 56° C before incubation with normal macrophages and schistosomules. Results are expressed as percentage of schistosomules with adherent macrophages.

was not restored by addition of guinea pig fresh serum. It was therefore assumed that a thermolabile factor could be responsible for macrophage adherence.

Absorption of rat immune sera by *S. mansoni* soluble antigens before incubation was shown to decrease significantly the adherence of the macrophage to the schistosomules, indicating that the factor was probably a thermolabile antibody. To assess this hypothesis, various experiments were devised.

The thermolability of the serum factor was confirmed by estimating the percentage of macrophage-coated schistosomules after incubation with sera heated for periods of 30–180 min (Fig. 2). Absorption of rat *S. mansoni* immune sera by increased quantities of goat anti-rat IgE serum, specific for ϵ chain, or $F(ab')_2$ of the same antiserum⁴, led

Table 1 Comparison of immune adherence of normal macrophages incubated in infected-rat serum before and after absorption with anti-rat IgE immunosorbent

Serum	% Schistosomules with adherent macrophages (mean $\pm$ s.d.)	Significance	IgE level (μ g ml ⁻¹) (mean $\pm$ s.d.)
Unabsorbed	86.25 $\pm$ 6.02	$C_1 = 3.26$ $P \leq 0.01$	9.70 $\pm$ 0.47
Day 59			
Absorbed	4.80 $\pm$ 3.56	$t = 25.455$ $P < 0.0001$	1.31 $\pm$ 0.32
Unabsorbed	92.25 $\pm$ 5.06	$C_1 = 3.26$ $P \leq 0.01$	10.20 $\pm$ 0.44
Day 68			
Absorbed	12.2 $\pm$ 9.70	$t = 29.371$ $P < 0.0001$	0.01 $\pm$ 0.40

The solid phase anti-IgE was prepared with goat-anti-rat IgE $F(ab')_2$ (ϵ specific) covalently linked to cyanogen bromide activated Sepharose. The rat serum was absorbed out by passage through the column, concentrated to the original volume and incubated with the normal rat macrophages. Means and s.d. of the percentages of coated schistosomules were calculated. Serum IgE determination was carried out by a radio-immunoassay; radioactivity bound was precipitated by 33% ammonium sulphate⁶.

to a progressive decrease of the macrophage adherence (Fig. 3).

Similar experiments with anti-rat IgG (IgG₁, IgG_{2a}, IgG_{2b}), IgM and IgA goat serum⁹ did not exhibit any significant decrease.

To avoid possible interference by soluble IgE-anti-IgE complexes and excess of anti-IgE antibodies, the same absorption experiment was carried out in solid phase using goat IgG anti-rat IgE linked to Cyanogen Bromide-activated Sepharose 3B. In these experimental conditions, a highly significant decrease of macrophage-coated schistosomules was observed (Table 1); the disappearance of IgE was controlled by radio-immunoassay.

The involvement of specific IgE antibodies in the immune adherence of normal macrophages to *S. mansoni* schistosomules could therefore be inferred. Further controls of specificity were performed with IgE myeloma serum (IR 162) (ref. 7) and with sera containing unrelated reaginic antibodies: anti-ragweed, anti-ovalbumin, anti-BGG-DNP and anti-*Nippostrongylus brasiliensis* with respective homologous PCA titres of 1:512, 1:256, 1:512, and 1:512, according to the technique of Jarrett and Ferguson⁸. None of these sera elicited any significant immune adherence of macrophages to schistosomules.

To investigate the mechanism of action of IgE in immune adherence, the possibility of nonspecific binding of IgE molecules on the macrophage surface was considered. Three different techniques were used. Rosette formation was obtained when normal rat macrophages incubated with rat IgE myeloma protein were put into contact with specific anti-rat IgE linked by glutaraldehyde on group O, rhesus negative, human red cells. Similar results were obtained when macrophages were incubated in serum from rats infected with *S. mansoni*.

Macrophages incubated with these immune rat sera demonstrated a fluorescent surface staining with goat serum anti-rat IgE revealed by anti-goat fluorescein-labelled globulins. No staining was obtained when the anti-*S. mansoni* rat serum was previously heated (56° C for 3 h) or absorbed by F(ab')₂ anti-rat IgE. The binding of IgE to the macrophage surfaces was confirmed by the use of peroxidase-labelled IgG anti-rat IgE and an ultrastructural study.

It seemed, therefore, that the nonspecific binding of IgE on rat macrophages resembles the cytophilic binding of some immunoglobulin classes in man and mouse^{9,10}. In our model the immune-adherence of the macrophage to schistosomules therefore seems to be the result on the one hand, of the specificity of IgE antibody against the parasite and, on the other hand, of a nonspecific binding on the macrophage surface, probably by the constant part of the IgE molecule.

Preliminary observations have indicated that the immune adherence of macrophages described here may have some importance in the killing of *S. mansoni* schistosomules *in vitro* methods¹¹, is highly accelerated if specific IgE antibodies for *S. mansoni* schistosomules, as studied by *in vitro* methods¹¹, is highly accelerated if specific IgE mediated adherence of normal macrophages occurs at the same time. Implications of these observations might be considered at two levels.

In the rat, reaginic antibodies were reported and their possible significance in *S. mansoni* infection considered^{12,13}. Our present observations bring an indication of a positive function of IgE antibodies in immunity to schistosomes in the rat.

Increased levels of IgE have been reported for a number of helminth infections in man¹⁴ and specific human IgE antibodies against schistosomes have now been demonstrated (J.-P.D., *et al.*, unpublished). It is reasonable then to consider the possible role of IgE-mediated macrophage adherence in schistosomiasis and other helminthoses of man.

To our knowledge, this is the first time that IgE antibodies

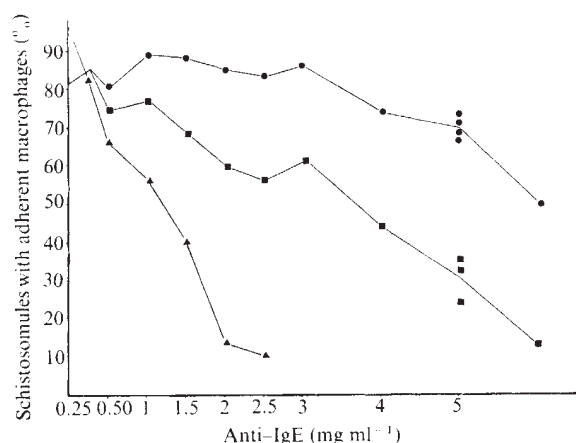


Fig. 3 Effect of absorption by increasing quantities of anti-rat IgE on adherence of incubated macrophages to *S. mansoni* schistosomules. A pool of group 1 rat sera was incubated with different anti-IgE concentrations (mg ml⁻¹), 2 h at 37° C, overnight at 4° C and centrifuged. Macrophages were incubated in supernatants. ▲, Absorption by goat IgG anti-rat IgE (ε specific); ■, absorption by F(ab')₂ prepared from goat IgG anti-rat IgE; ●, control experiment with F(ab')₂ prepared from normal goat IgG.

have been implicated in macrophage adherence reactions and the phenomenon should be investigated in other situations where specific IgE antibodies are involved. In any event, our experiments indicate that specific IgE anti-schistosome antibodies may have a central role in the immunity of the rat to this parasite, and are not merely concerned in the concurrent development of immediate hypersensitivity.

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- 1 Capron, A., in *Parasites in the Immunized Host: Mechanisms of survival* (CIBA Foundation Symposium, November 1973), 181-182 (Elsevier, Amsterdam, 1974).
- 2 Capron, M., *thesis*, (Lille, 1974).
- 3 Clegg, J. A., and Smithers, S. R., *J. Parasit.*, **56**, 56-57 (1970).
- 4 Bazin, H., Querinjean, P., Beckers, A., Heremans, J. F., and Dessy, H. *Immunology*, **26**, 713-723 (1974).
- 5 Bazin, H., Beckers, A., and Querinjean, P., *Eur. J. Immun.*, **4**, 44-48 (1974).
- 6 Carson, D., Metzger, H., and Bazin, H., *J. Immun.* (in the press).
- 7 Bazin, H., Beckers, A., Deckers, C., and Moriamé, M., *J. Natn. Cancer Inst.*, **51**, 1359-1361 (1973).
- 8 Jarrett, E., and Ferguson, A., *Nature*, **250**, 420-422 (1974).
- 9 Nelson, D. S., and Boyden, S. V., *Br. med. Bull.*, **23**, 15-10 (1967).
- 10 Berken, A., and Benacerraf, F., *J. exp. Med.*, **123**, 119-144 (1966).
- 11 Capron, A., Capron, M., Dupas, H., Bout, D., and Petitprez, A., *Int. J. Parasit.*, **4**, 613-623 (1974).
- 12 Ogilvie, B. M., Smithers, S. R., and Terry, R. J., *Nature*, **209**, 1221-1223 (1966).
- 13 Sadun, E. H., *Immunity to animal parasites*. (edit. by Soulsby E. J. L.), 97-129 (Academic Press, 1972).
- 14 Johansson, S. G. O., Bennich, H., and Berg, T., *Prog. clin. Immun.*, **1**, 157-190 (1972).

T-cell response to polyvinyl pyrrolidone is linked to maturity of B cells

HUMORAL antibody responses differ in their thymus dependency. Some antigens such as heterologous proteins¹, heterologous erythrocytes¹ and transplantation antigens² show a pronounced requirement for T helper cells, whereas other antigens such as bacterial polysaccharides^{3,4}, polymerised flagellin⁵ and certain synthetic polymers^{4,6} can induce a humoral antibody in the B lymphocytes in the absence of T helper cells. It has been suggested that the thymus-independent antigens are able to trigger the B cells directly because of their polymeric structure with repeating antigenic determinants⁷, because of their mitogenic properties for B cells⁸, or because they have the capacity to activate complement by means of an alternative pathway thus providing the C3 receptor-bearing B cells with an extra, activating signal⁹. It has also been proposed that thymus independency is only a relative, quantitative phenomenon and that some antigens seem thymus-independent since they need only extremely few T cells for interaction and triggering of the B cells¹⁰. It has also been demonstrated that thymus-independent antigens are slowly metabolised and non-degradable in the body, in contrast to the thymus-dependent ones⁶. The significance of this last observation is not known. We report some properties of the humoral antibody responses to polyvinyl pyrrolidone in mice. The thymus dependency of this response is shown to be a function of the stage of maturation of the B lymphocytes.

Inbred mice of (A/Sn × C57BL)F₁ genotype 8–12 months old were used as recipients in cell transfer experiments; animals at ages indicated below were used as cell donors. Immunisation with polyvinyl pyrrolidone (PVP; molecular weight 360,000) was carried out as previously described⁴ and serum antibody was determined by a haemolysis of PVP-labelled erythrocytes⁴. Preparation of cell suspensions and irradiation of mice was as previously described⁴.

Spleen cells from adult mice (2 months old) and young mice (2 weeks old) were compared with regard to their capacity to mount a humoral antibody response to PVP after transfer to lethally irradiated recipient mice. Table 1 shows that the adult spleen cells were able to produce antibody to this antigen, and that the addition of normal adult thymus cells markedly suppressed the antibody response. In contrast, we found that the young spleen cells produced only small amounts of antibody by themselves, the antibody response being enhanced by the addition of thymus cells from normal adult mice. The effects observed on the antibody production in the PVP system were the result of T cells residing in the cortisone-resistant part of the thymus,

Table 1 Helper or suppressor effect of thymus cells exerted on young or adult spleen B cells in their response to PVP

Spleen	Thymus*	Antibody response†
2 months, 2×10^7	—	3.9 ± 0.5
2 months, 2×10^7	10^7	1.8 ± 0.5
2 weeks, 2×10^7	—	2.2 ± 0.5
2 weeks, 2×10^7	10^7	4.1 ± 0.3

*Normal, 2 month old mice were used as thymus cell donors.

†Humoral antibody titre ($\log_2$) 2 weeks after lethal irradiation with 750 rad and intravenous infusion of cells together with 0.1 μ g PVP mixed with the cells. Each figure represents the mean from 18 animals $\pm$ s.e.m.

Table 2 Helper and suppressor effects of thymus cells. A comparison between thymus cells from normal, immune and tolerant animals

Thymus	Antibody titre*	
	Adult spleen	Young spleen
Normal	1.2 ± 1.2	4.5 ± 0.3
Immune†	1.9 ± 0.7	3.3 ± 0.4
Tolerant‡	5.6 ± 0.2	1.8 ± 0.9
No thymus cells given	5.6 ± 0.3	1.8 ± 0.8

*Humoral antibody titre ($\log_2$) in lethally irradiated mice 2 weeks after 750 rad together with intravenous infusion of the indicated cells with 0.1 μ g PVP. Each figure is the mean of five to six animals $\pm$ s.e. Animals had received 2×10^7 spleen cells, young or adult, either alone (as in the bottom group), or together with normal, immune or tolerant thymus cells in a dose of 10^7 .

†Immune thymus cell donors had received 0.1 μ g PVP 1 month earlier as an intraperitoneal injection.

‡Tolerant thymus cell donors had received 1,000 μ g PVP intraperitoneally 1 month earlier.

as the activity of the thymus cells was not affected by passage over anti-Ig columns, and the remaining thymus cells after cortisone treatment of the donor mice were still active (B. A. and H. B., unpublished). Thymus cells treated with mitomycin C were inactive, indicating that living T cells were needed for the effects (B. A. and H. B., unpublished). Comparing the spleen B cells from young and adult mice we found the same percentage (50–60%) of cells bearing Ig on their surface but the young spleen cells had only 2–17% C3 receptor-bearing cells compared to 21–56% in the adult spleens. This possibly indicates a role for the C3 receptor in determining thymus dependency. Depletion

Table 3 Maturing B cells from the bone marrow can be helped in anti-PVP antibody response by specifically immune thymus cells

Bone marrow	Thymus*	Antibody titre†
2×10^7	—	0.2 ± 0.2
2×10^7	10^7 normal	1.2 ± 1.2
2×10^7	10^7 tolerant	1.3 ± 0.6
2×10^7	10^7 immune	4.6 ± 0.4

*Normal, tolerant and immune thymus had received the same treatment as corresponding cell donor mice in Table 2.

†Humoral antibody titres in lethally irradiated mice (750 rad) receiving the indicated cells intravenously together with 0.1 μ g PVP. Animals were bled 11 days after irradiation and cell transfer. Each group consisted of five to ten mice.

of C3-bearing cells by EAC columns¹¹ or EAC rosette sedimentation¹² and further studies on the thymus dependency in the depleted populations may provide the answer.

The effects of thymus cells on the humoral antibody response to PVP could be specific, mediated by T lymphocytes recognising antigenic determinants on the PVP molecules and acting as specific helper or suppressor cells. The effects of the thymus cells could, however, also be nonspecific, mediated, for example, by the release of factors inhibiting or stimulating the rate of proliferation and/or maturation of the B cells. To discriminate between these possibilities, the activities of thymus cells from normal, immune and tolerant donor mice were compared. Table 2 shows that pretreatment of the thymus donor mice with high doses of PVP, capable of producing tolerance, abolishes the suppressing and the helping activities of the thymus cells, whereas normal and immune thymus cells exerted both suppressing and helping activities. Table 3 shows that immune thymus cells actually perform better in one respect

than normal and tolerant thymus cells, namely in their capacity to act as helper cells for maturing B cells early after lethal irradiation and bone marrow reconstitution.

Table 1 shows that, when 2×10^7 spleen cells and 10^7 thymus cells are given, young spleen cells are stimulated and adult spleen cells are suppressed in their anti-PVP response. We point out, however, that these effects are observed only at certain critical ratios between spleen cells and thymus cells. Table 4 shows that the suppressing effect of thymus cells is not present when lower or higher doses of thymus cells than 10^7 are used to modify the response of 2×10^7 adult spleen cells. One possibility is that stimulation and suppression are exerted by different subpopulations of immunocompetent cells present in the thymus, and that at high cell doses the suppressor cell activity is counteracted by helper cells. It may also be that heterogeneity exists in the adult spleen B cell population and that subpopulations

of immature B cells and therefore the effects may be of the same kind as those we report.

It is still not known whether the enhancing and suppressing activities of T cells on antigens like PVP are mediated by the same or by different subsets of T cells or thymus cells. In either case, the possibility of detecting helper cell activity or suppressor cell activity, evident from our data, depends on the stage of maturation of the B cells used in the experiments.

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Table 4 Helper and suppressor effects of thymus cells: variations with cell dose

Normal thymus	Antibody titre*	
	Adult spleen	Young spleen
—	3.8 ± 0.2	1.0 ± 0.5
4×10^7	2.6 ± 0.7	0.9 ± 0.6
2×10^7	4.0 ± 0.0	1.0 ± 0.5
10^7	$0.0 (< 1.0)$	3.4 ± 0.3
5×10^6	4.4 ± 0.2	3.7 ± 0.2
2×10^6	4.2 ± 0.5	3.8 ± 0.4
10^6	3.3 ± 0.8	ND

*Experimental conditions as in Table 2. For adult spleen, five recipient mice per group; for young spleen, eight to ten mice per group. ND, not determined.

of B cells with different requirements for helper T cells exist. The young spleen cells can be stimulated by relatively small doses of thymus cells and when the thymus cell dose is increased over a critical level (10^7 thymus cells for 2×10^7 young spleen cells) suppression is taking place. This could be due to heterogeneity of thymus cells, that is, different subsets of cells exerting suppression and stimulation. One population of T cells exerting both functions but effective at different optimal concentrations would give the same picture.

The humoral antibody response to PVP was found to be thymus-independent in adult thymectomised, lethally irradiated and bone marrow-reconstitution mice 3–4 weeks after the reconstitution, when presumably the B cells were as mature as those of normal adult mice⁴. We would, therefore, like to modify the concept of thymus independence for this antigen in the following way. Mature B cells respond to PVP without T-cell help. Under certain experimental conditions T cells may instead suppress the response, as evidenced from numerous publications of suppressor T cells during the past few years in a variety of systems¹³ including the anti-PVP system of mice^{14–16}. Immature B cells from young mice require T cells as helpers to respond optimally to PVP. There is at least one previous publication indicating that the concept of T cell dependency in immature B cells may be a more general phenomenon: it has been reported that the B cells from Peyer's patches in mice are poorly stimulated by the B-cell mitogen *E. coli* lipopolysaccharide¹⁷ and that the mitogenic response is enhanced by the addition of T cells. The Peyer's patches may contain a high propor-

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- 1 Miller, J. F. A. P., Mitchell, G. F., Davies, A. J. S., Claman, H. N., Chaperon, E. A., and Taylor, R. B., *Transplant. Rev.*, **1**, 1–149 (1969).
- 2 Klein, J., Livnat, S., Hauptfeld, V., Jerabek, L., and Weissman, I., *Eur. J. Immunol.*, **4**, 41–44 (1974).
- 3 Humphrey, J. H., Parrott, D. M. V., and East, J., *Immunology*, **7**, 419–434 (1964).
- 4 Andersson, B., and Blomgren, H., *Cell. Immunol.*, **2**, 411–424 (1971).
- 5 Feldman, M., and Basten, A. J., *Expl. Med.*, **134**, 103–119 (1971).
- 6 Sela, M., Mozes, E., and Shearer, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2696–2700 (1972).
- 7 Moller, G., in *Immune Surveillance*, 112–116 (Academic, New York, 1970).
- 8 Coutino, A., and Moller, G., *Scand. J. Immunol.*, **3**, 133–146 (1974).
- 9 Dukor, P., and Hartmann, K. U., *Cell. Immunol.*, **7**, 349–356 (1973).
- 10 Bretscher, P., *Transplant. Rev.*, **11**, 217–267 (1972).
- 11 Wigzell, H., Huber, C., and Schirmacher, V., *Haematologica*, **6**, 369–375 (1972).
- 12 Jondal, M., Wigzell, H., and Aiuti, F., *Transplant. Rev.*, **16**, 163–195 (1973).
- 13 Gershon, R. K., in *Contemporary Topics in Immunobiology* (edit. by Cooper, M. D., and Warner, N. L.) **3**, 1–40 (Plenum, New York, 1974).
- 14 Kerbel, R. S., and Eidinger, D., *J. Immunol.*, **106**, 917–926 (1971).
- 15 Kerbel, R. S., and Eidinger, D., *Eur. J. Immunol.*, **2**, 114–118 (1972).
- 16 Rotter, V., and Trainin, N., *Cell. Immunol.*, **13**, 76–86 (1974).
- 17 Kagnoff, M. F., Billings, P., and Cohn, M., *J. exp. Med.*, **139**, 407–413 (1974).

Absence of age-resistance in neonatally thymectomised chickens as evidence for cell-mediated immune surveillance in Marek's disease

MAREK's disease virus (MDV) of chickens induces lymphomas and other lymphoproliferative changes, which, depending on the virulence of the strain used, may terminate in paralysis and death. Chickens genetically selected for resistance, however, are refractory to clinical MD and most chickens that are fully susceptible when young develop resistance as they become older. Resistance acquired with age is expressed through lesion regression^{1,2}. Our studies with genetic and age resistance established that humoral immunity did not play a major role in either type of resistance^{3,4} and now we present evidence that the thymus-dependent immune system is of principal importance in the natural resistance of old chickens.

Two experiments were conducted. White Leghorn chickens of cross 15 × 7 with maternal MDV antibody were either thymectomised (TX) or TX and treated with cyclophosphamide (CY, Cytosan, Mead Johnson Laboratories, Evansville, Indiana) at hatching. These and appropriate control groups were raised in isolation until 8 weeks old; and then inoculated intra-abdominally with cell-associated MDV (JM strain). Before virus inoculation, all groups were tested⁵ and found to be free from adventitious infection with MDV. Chickens were observed for 12 and 14 weeks after inoculation, in the first and second experiments respectively. Chickens dying during the observation period and those surviving at the termination of the experiments were necropsied and if gross lesions of MD were absent, peripheral nerves and gonads were examined for histological lesions⁶. The survivors were also tested for MDV antibody by the agar gel precipitin test. In TX groups, only the chickens that lacked grossly detectable thymus remnants were considered adequately TX and included in the data.

Table 1 T-cell and B-cell functions in chickens thymectomised (TX) and thymectomised plus cyclophosphamide (CY) treated at hatching (experiment 2)

Treatment at hatching	No. of chickens	Thymus remnants	Delayed hypersensitivity +/tested	Sheep erythrocytes (Haemagglutination test)		Antibody to* <i>B. abortus</i> (Plate agglutination test)		MDV (agar precipitin test) +/tested
				+ / tested†	Mean titre (log ₂)‡	+ / tested	Mean titre (log ₂)	
γ-radiation	16	Present	15/16	15/15	6.9	15/15	2.5	16/16
TX + γ-radiation	9	Absent	4/9	9/9	7.4	9/9	3.4	2/2
TX + γ-radiation + CY	11	Absent	3/11	0/11	0.0	0/11	0.0	0/2
CY treated	15	Present	15/15	10/15§	3.7	1/15	3.0	0/14
None	13	Present	13/13	13/13	8.8	12/13	1.8	13/13

* All groups were inoculated intravenously with sheep erythrocytes and *B. abortus* (2.5×10^8 cells of each) at 5 and 7 weeks of age and with MDV (intra-abdominally) at 8 weeks. Antibody to sheep erythrocytes and *B. abortus* was determined just before MDV inoculation. Tests for MDV antibody were done at the end of the experiment.

† Titre of $\geq 1:4$ was considered positive.

‡ Highest dilution tested was 1:512.

§ Five chickens negative by the haemagglutination test were also negative for antibody against *B. abortus*.

Chickens were thymectomised surgically⁵ within 30 h of hatching. On the day following surgery, TX and appropriate control chickens were exposed to 600 rad of total body γ radiation (88 rad min⁻¹ at 2 m from cobalt-60, 24,000 Ci, Michigan State University). Thymus function was tested by delayed-type hypersensitivity (DTH) reaction. Chickens were sensitised with complete Freund's adjuvant and 4 weeks later, tested for local reaction to intradermal injection of old tuberculin into the right wattle⁶.

Deficiency in the B-cell function was induced with 16 mg of CY given in four daily injections, each of 4 mg, commencing 2 d after hatching. In the CY-treated groups, only chickens that failed to produce antibody against sheep erythrocytes, *Brucella abortus* and MDV were included in the data (Table 1).

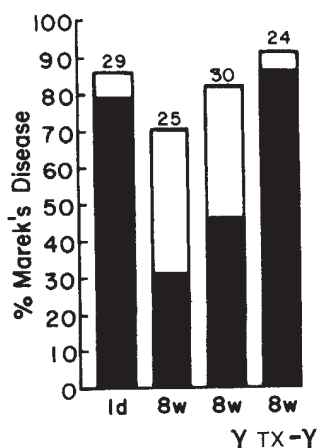
In the first experiment all survivors in MDV-inoculated but none in uninoculated control groups had MD antibody at termination. As Fig. 1 shows, the group exposed at 8 weeks had a significantly lower incidence of gross lesions and mortality than the group exposed at 1 d ($P < 0.01$, χ^2 test), thus demonstrating age resistance in the older chickens. As observed earlier^{1,2}, however, age resistance was not well pronounced at the level of microscopic lesions. The incidence of gross lesions and mortality in the γ-irradiated older group was slightly higher, although not significantly ($P > 0.5$), than that in untreated 8-week-old chickens. Like the unirradiated chickens, the irradiated chickens had a significantly lower incidence of gross lesions and mortality than that in the 1-d-old group

($P < 0.01$) thus illustrating the presence of age resistance. In the TX group, 24 chickens lacked thymus remnants at necropsy: 13 of these were negative for DTH. Among the 24 TX chickens, the incidence of mortality and gross lesions was 87%; significantly higher than that in the untreated ($P < 0.01$) and γ-irradiated 8-week-old groups ($P < 0.01$). In the second experiment (Fig. 2) the resistance of 8-week-old chickens was more pronounced than in the first. Incidence of death and gross lesions was 15%, whereas incidence in the 1-d-old group was 90%. γ Irradiation did not significantly influence the resistance of older birds. The thymus- and bursa-dependent functions of various treatment groups in the second experiment are given in Table 1. Of the 15 CY treated chickens, five were free of antibody against sheep erythrocytes and *B. abortus*. Four of the five were resistant, however, confirming that humoral immunity alone does not play an important role in age resistance to MD⁴. All 9 chickens in the TX and 11 in the TX + CY treated groups either died as a result of MD or had gross lesions at termination. These results were consistent with the results of the first experiment. Because CY treatment alone did not influence resistance, the lack of resistance in the TX + CY treated group was attributed to thymectomy. The incidence of microscopic lesions in older age groups was lower than in the first experiment probably because chickens were held longer and more lesions regressed.

The effect of the absence of T cells on MDV has not previously been reported, presumably because T-cell function cannot be completely obliterated with available surgical procedures. In our experiments also, thymectomy failed to eliminate all T-cell function because many TX birds, although lacking in detectable thymus remnants, reacted in DTH to old tuberculin. T-cell function was, however, definitely suppressed to significantly alter the MD response.

The results of this study also have important implications for the understanding of the pathogenesis of MD. The mechanism by which lymphoid cells are triggered to proliferate into lymphomas is not known. Because most of the lymphoma cells in MD are of T origin^{7,8} and because functional bursectomy does not influence lymphoma formation^{9,10}, it has been proposed that the target cell, that is the cell that undergoes neoplastic transformation, is of thymic origin. Several lymphoid cell lines have been developed from MD lymphomas¹¹⁻¹³ and the cells of these lines bear T-cell surface markers^{13,14}. In the light of this evidence for the T cell being the target cell for MD virus the high incidence of gross lesions in the TX birds in our experiments was unexpected. Our results can, however, be explained if, as stated above, neonatal thymectomy did not eliminate all T cells and the TX birds had enough remnant T cells to serve as the target for MDV, yet insufficient to mount a satisfactory cell-mediated immune response. The residual T cells may have originated from thymic remnants¹⁵ or from cells that had left the thymus before thymectomy. Nonetheless, our results indicate a pressing need for a more direct identification of the target cell in MD. Recognition of specific tumour

Fig. 1 The effect of neonatal thymectomy (TX) and γ-radiation (γ) on age resistance to MDV. Numbers below columns indicate the age in days (d) or weeks (w) at the time of MDV inoculation (1.0×10^4 PFU per chicken). Numbers above columns indicate the number of chickens in each group. Total MD includes chickens with histological lesions at termination of the experiment. Black indicates mortality plus gross lesions and white indicates total MD.



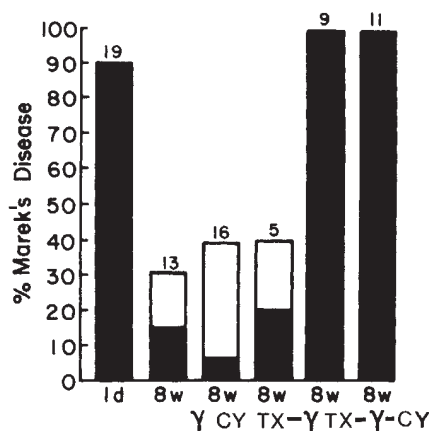


Fig. 2 The effect of neonatal thymectomy (TX) and γ -radiation (γ) and of TX- γ and cyclophosphamide (CY) treatment on age resistance to MDV inoculation (5.5×10^4 PFU per chicken). See legend to Fig. 1.

antigens¹³ may facilitate detection of transformation *in vivo*.

If T cells acted as target cells, the presence of the thymus was apparently not necessary for initial virus-cell interaction and for emergence of transformed clones. Because gross lesions were found in most chickens without thymus remnants, the initial transformation probably occurred in extra thymic T cells. This situation is in contrast with that of lymphoid leukosis in chickens where virus-induced transformation of target cells originates in B cells within the physical confines of the bursa of Fabricius and the removal of this organ, even well after many B cells have peripherised, prevents tumour formation^{16,17}.

We concluded that age resistance and hence lesion regression in MD is mediated through thymus-dependent cellular immune functions.

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- Witter, R. L., Sharma, J. M., Solomon, J. J., and Champion, L. R., *Avian Pathol.*, **2**, 43 (1973).
- Sharma, J. M., Witter, R. L., and Burmester, B. R., *Infect. Immun.*, **8**, 715 (1973).
- Sharma, J. M., *Nature*, **247**, 117 (1974).
- Sharma, J. M., and Witter, R. L., *Cancer Res.* (in the press).
- Peterson, R. D. A., Burmester, B. R., Fredrickson, T. N., Purchase, H. G., and Good, R. A., *J. natn. Cancer Inst.*, **32**, 1343 (1964).
- Cooper, M. D., Peterson, R. D. A., South, M. A., and Good, R. A., *J. exp. Med.*, **123**, 75 (1966).
- Hudson, L., and Payne, L. N., *Nature new Biol.*, **241**, 52 (1973).
- Rouse, B. T., Wells, R. J. H., and Warner, N. L., *J. Immun.*, **110**, 543 (1973).
- Payne, L. N., and Rennie, M., *J. natn. Cancer Inst.*, **45**, 387 (1970).
- Fernando, W. W. D., and Calnek, B., *Avian Diseases*, **15**, 467 (1971).
- Akiyama, Y., Kato, S., and Iwa, N., *Biken J.*, **16**, 177 (1973).
- Akiyama, Y., and Kato, S., *Biken J.*, **17**, 105 (1974).
- Powell, P. C., Payne, L. N., Frazier, J. A., and Rennie, M., *Nature*, **251**, 79 (1974).
- Nazerian, K., and Sharma, J. M., *J. natn. Cancer Inst.* (in the press).
- Panigrahi, D., Waxler, G. L., and Mallman, V. H., *J. Immun.*, **107**, 289 (1971).
- Peterson, R. D. A., Purchase, H. G., Burmester, B. R., Cooper, M. D., and Good, R. A., *J. natn. Cancer Inst.*, **36**, 585 (1966).
- Cooper, M. D., Payne, L. N., Dent, P. B., Burmester, B. R., and Good, R. A., *J. natn. Cancer Inst.*, **41**, 373 (1968).

Effect of ascorbic acid on hyaluronidase inhibitor

THE presence of a physiological inhibitor of hyaluronidase (PHI) in mammalian serum was suggested by Duran-Reynals¹ who observed the destruction of spreading factor by blood. Hobby *et al.*² and McClean³ later reported the inhibition of microbial and mammalian hyaluronidase by serum. PHI, which is a glycoprotein and sensitive to heat⁴,

has been shown to increase in both bacterial and viral infections, cancer and various liver, kidney and rheumatic diseases^{4,5}. A small increase of PHI has been reported in scorbutic guinea pigs by Shack *et al.*⁶. These increases have been suggested^{5,8} to represent a defence against invasive microorganisms, or a non-specific response to tissue inflammation or destruction⁴. (Fischer-Szafarz⁷ described a hyaluronidase inhibitor in the serum of cancer patients which she believes is different from PHI.) Cameron and Pauling suggested that a high intake of ascorbic acid increases the biosynthesis of PHI, resulting in a decrease in hyaluronidase activity⁹. Consequently, the invasiveness of proliferative diseases would be prevented by reduced depolymerisation of the intercellular matrix of the proliferating cells. We have investigated the effect of doses of ascorbic acid of 0–1,000 mg kg⁻¹ d⁻¹ on levels of PHI in the serum of guinea pigs. Our results show that PHI is not affected by high levels of dietary ascorbic acid.

Guinea pigs receiving 20, 200, 400 and 1,000 mg kg⁻¹ d⁻¹ gained weight at identical rates throughout the experiment. Those animals which received no ascorbic acid lost weight after 14 d and developed scurvy (Fig. 1).

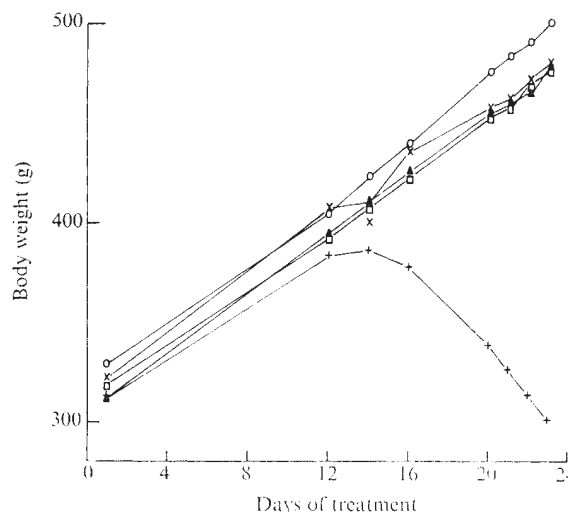


Fig. 1 Effect of ascorbic acid on body weight. Adult male Hartley guinea pigs were fed a vitamin C-deficient Reid Briggs diet and received varying amounts of ascorbic acid in drinking water for 22–23 d. Based on an average daily water consumption of 10 ml per 100 g body weight, the animals received 0, 20, 200, 400 or 1,000 mg kg⁻¹ d⁻¹. Body weights were measured periodically. +, Untreated; ○, 20 mg kg⁻¹ d⁻¹; ×, 200 mg kg⁻¹ d⁻¹; ▲, 400 mg kg⁻¹ d⁻¹; □, 1,000 mg kg⁻¹ d⁻¹. Each point represents the mean of 15 animals.

In experiment 1 the serum from each animal was assayed individually for PHI by the hyaluronidase turbidity assay (Table 1). Scorbutic guinea pigs had significantly more PHI ($P < 0.02$) than those receiving 20 mg kg⁻¹ d⁻¹ and there was no significant difference in levels of PHI among any of the groups receiving ascorbic acid. In experiment 2 (Table 1) sera from 15 animals were pooled in groups and assayed for PHI. Again the levels of PHI in the scorbutic group were significantly ($P < 0.01$) higher than in animals receiving ascorbic acid. Again there was no significant difference in PHI levels in the groups receiving ascorbic acid. Sera from animals in experiment 1 were then pooled and assayed for PHI using ³⁵S-chondroitin sulphate (ChS) as a substrate for hyaluronidase (Fig. 2). The greatest inhibition of hyaluronidase activity was achieved with sera of scorbutic animals. Animals receiving 200 or 400 mg kg⁻¹ d⁻¹ of ascorbic acid did not have a greater level of PHI than those receiving 20 mg kg⁻¹ d⁻¹.

Since scorbutic guinea pigs consume less food than normal animals, we investigated the effect of this decreased food

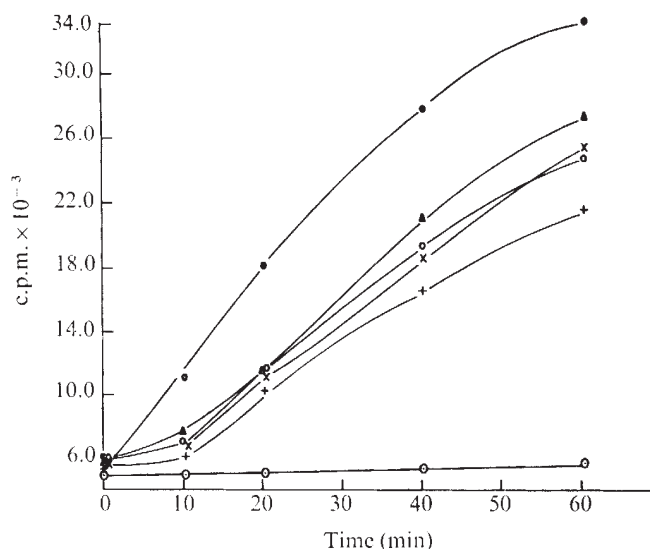


Fig. 2 Hydrolysis of ^{35}S -chondroitin sulphate by hyaluronidase. ●, Hyaluronidase control; +, hyaluronidase preincubated with sera from untreated animals; ○, hyaluronidase preincubated with sera from animals treated with 20 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$; ×, hyaluronidase preincubated with sera from animals treated with 200 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$; ▲, hyaluronidase preincubated with sera from animals treated with 400 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$; ○, boiled hyaluronidase. The conditions for preincubation of the PHI and hyaluronidase were identical with those described for Table 1, except that 6.0 ml of enzyme was preincubated with 0.2 ml of guinea pig serum. After preincubation 3.0 ml of the hyaluronidase-PHI mixture was added to 3.0 ml of 0.3 M phosphate buffer, pH 5.5, containing 13 μg of ^{35}S -chondroitin sulphate (800,000 c.p.m.). The reaction was maintained at 37°C for 1 h. Samples (1 ml) were removed at the designated times and added to 1 ml of 2% cetylpyridinium chloride. After 2 h at room temperature, the precipitate was centrifuged, and 0.5 ml of supernatant was placed in 10 ml of Aquasol, and counted in a Packard liquid scintillation counter. The ^{35}S -chondroitin sulphate was isolated from costal cartilage which had been incubated in Krebs-Ringer bicarbonate containing 0.2 mCi of carrier free $\text{H}_2^{35}\text{SO}_4$.

Table 1 Effect of ascorbic acid on serum PHI

Ascorbate intake (mg $\text{kg}^{-1} \text{d}^{-1}$)	Experiment	Hepatic ascorbate (mg per 100 g tissue)*†	Inhibitor*‡ (U)
0	1	1.43 ± 0.23	37.2 ± 11.2
	2	1.21 ± 0.59	40.8 ± 6.4
20	1	11.30 ± 5.43	25.2 ± 12.0
	2	10.55 ± 1.96	32.0 ± 4.0
200	1	30.65 ± 9.54	27.6 ± 8.0
	2	23.53 ± 3.76	28.8 ± 4.0
400	1	31.86 ± 4.30	24.4 ± 8.0
	2	23.89 ± 3.58	32.8 ± 5.2
1,000	2	23.31 ± 7.02	33.6 ± 3.2

In experiment 1 PHI units were measured in the serum of each of the 15 animals in each group. In experiment 2 they were measured in sera pooled from the 15 animals in each group, and each serum pool was assayed nine times.

*Each value represents the mean ± standard deviation.

†Hepatic ascorbic acid was determined in each animal by the method of Roe *et al.*¹⁰.

‡Blood was withdrawn on the last day of each study by cardiac puncture, and serum PHI was determined according to the method of Dorfman *et al.*¹¹ with the following modifications. Guinea pig serum (25 μl) was added to 1.0 ml of solution containing 2.5 μg of bovine testicular hyaluronidase (Worthington), 0.02 M phosphate buffer, pH 7.0, 0.001 M MgCl_2 , 0.45% NaCl and 0.01% bovine serum albumin. The hyaluronidase-PHI mixture was incubated for 10 min at room temperature. After 10 min, 0.5 ml of the mixture was added to 0.5 ml of 0.3 M phosphate buffer, pH 5.5, containing 0.2 mg of hyaluronidase. The reaction was maintained at 37°C for 40 min, and then terminated by the addition of 5.0 ml of acid albumin. The resulting turbidity was allowed to develop for 10 min before reading at 600 nm on a Zeiss spectrophotometer.

intake as a factor in increased PHI in scorbutic animals. PHI and hepatic ascorbic acid were assayed in animals receiving 20 mg $\text{kg}^{-1} \text{d}^{-1}$ of ascorbic acid by oral intubation and then fasted for 0, 24, 48, or 72 h. There was little or no change in PHI or ascorbic acid as a result of fasting.

There can be a tenfold increase in serum PHI in certain diseases¹. Table 1 and Fig. 2 show a modest but significant increase in the PHI levels of scorbutic animals compared with those receiving 20 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$.

These observations confirm the results of Shack *et al.*⁶ who showed an increase of approximately 40% in the PHI of scorbutic guinea pigs compared with animals on a normal diet. Our results do not support the hypothesis of Cameron and Pauling⁹ that ascorbic acid may ameliorate proliferative diseases by increasing biosynthesis of PHI. We did not find any significant change in the PHI of animals receiving 20–1,000 mg ascorbic acid $\text{kg}^{-1} \text{d}^{-1}$.

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- 1 Duran-Reynals, F., *J. exp. Med.*, **58**, 161–181 (1933).
- 2 Hobby, G. L., Dawson, M. H., Meyer, K., and Chaffee, E., *J. exp. Med.*, **73**, 109–123 (1944).
- 3 McClean, D., *J. Path. Bact.*, **54**, 284–286 (1942).
- 4 Mathews, M. B., and Dorfman, A., *Physiol. Rev.*, **35**, 381–402 (1955).
- 5 Glick, D., *J. Mt. Sinai Hosp.*, **17**, 207–228 (1950).
- 6 Shack, J. A., Whitney, R. W., and Freeman, M. E., *J. biol. Chem.*, **184**, 551–555 (1950).
- 7 Fiszler-Fzafray, B., *Proc. Soc. exp. Biol. Med.*, **129**, 300–302 (1968).
- 8 Haas, E., *J. biol. Chem.*, **163**, 63–89, 101–110 (1946).
- 9 Cameron, E., and Pauling, L., *Oncology*, **27**, 181–192 (1973).
- 10 Roe, J. H., Mills, M. B., Oesterling, M. J., and Damron, C. M., *J. biol. Chem.*, **174**, 201–208 (1948).
- 11 Dorfman, A., Ott, M. L., and Whitney, R., *J. biol. Chem.*, **174**, 621–629 (1948).

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matters arising

High affinity uptake of neurotransmitter amino acids

LEVI and Raiteri¹ have concluded that their results on the uptake and release of ³H-GABA by rat brain synaptosomes indicate that homoeexchange is a major mechanism by which radioactive GABA enters synaptosomes when low substrate concentrations are used. They therefore question the effectiveness of high affinity uptake mechanisms in terminating the postsynaptic actions of this and other neurotransmitter amino acids after their release from pre-synaptic nerve terminals in the central nervous system (CNS)^{2,3}.

I believe that their conclusion is misleading. It is based largely on two findings. First, that unlabelled GABA or glycine in low concentrations stimulate the efflux of ³H-GABA or ³H-glycine from synaptosomes previously labelled with these amino acids. These experiments were conducted, however, with synaptosomes that had been incubated for 10 min in 0.5 μ M labelled amino acid; from our own experience with such preparations we have found that the accumulation of tritiated amino acid is by this time approaching a steady state, in which the rate of influx is approximately equal to the rate of efflux, that is, a homoeexchange situation may exist. Addition of exogenous unlabelled amino acid in these conditions would therefore be expected to lead to the increased efflux of labelled amino acid observed by Levi and Raiteri¹.

These results do not contradict the hypothesis that the initial rate of influx of labelled GABA or glycine into synaptosomes or brain slices incubated *in vitro* represents a net accumulation of amino acid in the intracellular space. Previous observations⁴ by Levi and Raiteri and our own results⁵ have shown the initial rate of influx of labelled GABA into synaptosomes or brain slices is unaffected either by large increases in the size of the intracellular GABA pool induced by previous incubation in a medium containing a high concentration of the amino acid, or by treatment of animals with the GABA-glutamate transaminase inhibitor amino-oxyacetic acid. These findings are hard to reconcile with the notion that the accumulation of labelled

GABA occurs largely by homoeexchange, as if this were so the initial rate of influx of labelled GABA should be increased in such conditions.

Second, Levi and Raiteri measured the net removal of GABA from the incubation medium when synaptosomes were incubated with ³H-GABA at concentrations from 1 to 10 μ M. They show that the net uptake of amino acid is less than that expected from measurements of the removal of radioactivity from the medium, although approximately 40% of the total radioactivity removed could be accounted for by a net accumulation of GABA. These results, however, are in complete disagreement with those of similar experiments from the same laboratory⁴ which showed that net uptake of GABA accounted for more than 95% of the uptake of ³H-GABA in synaptosome preparations from various regions of rat CNS incubated with 20 μ M ³H-GABA. Aprison and McBride also observed a net accumulation of glycine in rat spinal cord synaptosomes when these are incubated with low concentrations of glycine (37.5–150 μ M).

The results of Levi and Raiteri also disagree with our previous findings² which showed that a net uptake of GABA accounted for more than 80% of the total uptake of ³H-GABA in rat brain slices incubated with 200 μ M GABA. In these conditions the uptake of exogenous GABA led to more than a threefold increase in the total GABA content of the tissue.

Nevertheless, after a 30 min incubation with this relatively high concentration of exogenous GABA there was also a significant exchange between the endogenous GABA content of the tissue and ³H-GABA in the external medium². Such exchange is not unexpected and does not contradict the conclusion that high affinity transport mechanisms for amino acids are capable of a net removal of amino acid from the external medium, and can thus represent important mechanisms for terminating the actions of these pharmacologically active substances in the CNS.

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- ¹ Levi, G., and Raiteri, M., *Nature*, 250, 735 (1974).
- ² Iversen, L. L., and Neal, M. J., *J. Neurochem.*, 15, 1141 (1968).
- ³ Logan, W. J., and Snyder, S. H., *Brain Res.*, 42, 413 (1972).
- ⁴ Levi, G., Bertollini, A., Chen, J., and Raiteri, M., *J. Pharmac. exp. Ther.*, 188, 429 (1974).
- ⁵ Snodgrass, S. R., and Iversen, L. L., *J. Neurochem.*, 20, 431 (1973).
- ⁶ Aprison, M. H., and McBride, W. J., *Life Sci.*, 12, 449 (1973).

LEVI AND RAITERI REPLY—When synaptosomes are incubated in a definite volume of medium containing a very low ³H-GABA concentration, the steady-state accumulation that is approached after a few minutes does not correspond to a saturation of the synaptosomal uptake capacity, but is a relative value which depends on the concentration of GABA in the medium and on the ratio between the amount of tissue and the volume of medium. If the same amount of synaptosomes is either incubated in a larger volume of medium or, even more so, if it is superfused with this medium, uptake continues to increase for a much longer period. Moreover, similar uptakes of 10 and 20 μ M ³H-GABA were obtained in synaptosomes that had been preincubated in the presence or in the absence of 0.5 μ M unlabelled GABA, and similar results were obtained in superfusion exchange experiments when synaptosomes were preincubated in 0.05 μ M ³H-GABA instead of 0.5 μ M. For these reasons the synaptosomes used in our experiments¹ must be considered as prelabelled, and not as preloaded to an "absolute" steady state.

If one accepts this concept, and assumes, as is generally done, that the labelled GABA mixes with the endogenous pool, then our results do show that homoeexchange is an important mechanism by which labelled GABA is accumulated by synaptosomes, particularly when low GABA concentrations are used. As homoeexchange has never been considered to contribute significantly to the initial rate of uptake of radioactive GABA by nerve terminals, in our opinion it is not possible, at present, to say whether the net uptake of this amino acid (which we did not deny) has or has not a high affinity component (K_m between 4×10^{-7} M (ref. 2) and 2×10^{-5} M (ref. 3)). We suggest that, even if a high affinity component did not exist, a low affinity uptake system (K_m of the order

of 10^{-3} M) might still be effective for the synaptic inactivation of GABA. The data of Fonnum *et al.*⁴, who estimated the concentration of GABA in 'gabergic' nerve terminals to be "at least 60 mM, probably over 100 mM", seem to support this suggestion.

As to the other comments raised, we are obviously aware of the fact that our present data disagree with previous experiments conducted in our own as well as in other laboratories. This did not seem to us a strong enough reason not to publish the data. Moreover, the disagreement is often more apparent than real, and in most cases concerns the interpretation of the data rather than the data themselves. For example, the finding³ that cortex slices (which contain structures other than nerve endings) show a net uptake of GABA after 30 min of incubation in the presence of a concentration (200 μ M) at which high affinity uptake^{3,5,6} and exchange¹ are virtually saturated, does not disprove that homoeexchange can account for a large part of the initial rate of accumulation of radioactivity by synaptosomes incubated with 1 or 10 μ M ³H-GABA.

Similarly, our data on glycine (detectability of homoeexchange in spinal but not in cortical synaptosomes) are not disproven by those of Aprison and McBride⁷ who did show a net uptake of unlabelled glycine, but did not assess the contribution of exchange to the accumulation of radioactive glycine. Moreover, these authors may have somewhat overestimated net uptake, as they did not consider the increase in glycine concentration that might have occurred in the tissue on incubation without added glycine⁸.

The similarity between radiochemical and chemical uptake of 20 μ M GABA that we found in a previous study⁹ is difficult to explain at present. In this type of experiment the choice of a correct type of control is very critical, and we are now trying to determine whether an overestimation of net uptake might have resulted from the type of control chosen.

The results of the experiments in which the tissue concentration of GABA was artificially increased^{9,10} could have several explanations: for example, the increased intracellular concentration of GABA may cause a concomitant inhibition of net uptake and a stimulation of homoeexchange. In appropriate experimental conditions we have shown that the accumulation of radioactive GABA is increased in synaptosomes prepared from amino-acetic acid-treated rats.

We take this opportunity to mention that one of the data reported in Fig. 2 of our letter¹ was wrong, because of a trivial technical error. The figure showed a rapid and massive depletion of synaptosomal GABA content and

the absence of any detectable homoeexchange on superfusion with a sodium-free medium. Our latest data (Raiteri *et al.*, unpublished) indicate that superfusion with sodium-free medium (NaCl replaced by sucrose) does not cause a significant increase in the spontaneous release of ³H-GABA from purified synaptosomes. They do confirm, however, that, in the absence of sodium—homoeexchange is 95–100% inhibited. Therefore, the error does not invalidate the conclusion that absence of homoeexchange could account for what looks like inhibition of the high affinity uptake of GABA.

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- ¹ Levi, G., and Raiteri, M., *Nature*, **250**, 735 (1974).
- ² Henn, F. A., and Hamberger, A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2686 (1971).
- ³ Iversen, L. L., and Johnston, G. A. R., *J. Neurochem.*, **18**, 1939 (1971).
- ⁴ Fonnum, F., Grofovà, I., Rinvik, E., Storm-Mathisen, J., and Walberg, F., *Brain Res.*, **71**, 77 (1974).
- ⁵ Iversen, L. L., and Neal, M. J., *J. Neurochem.*, **15**, 1141 (1968).
- ⁶ Levi, G., and Raiteri, M., *Life Sci.*, **12** (1), 81 (1973).
- ⁷ Aprison, M. H., and McBride, W. J., *Life Sci.*, **12**, 449 (1973).
- ⁸ Bradford, H. F., and Thomas, A. J., *J. Neurochem.*, **16**, 1495 (1969).
- ⁹ Levi, G., Bertollini, A., Chen, J., and Raiteri, M., *J. Pharmac. exp. Ther.*, **188**, 429 (1974).
- ¹⁰ Snodgrass, S. R., and Iversen, L. L., *J. Neurochem.*, **20**, 431 (1973).

Playing possum

IN his interesting article on the opossum α haemoglobin chain sequence, Stenzel¹ states that 'a selectionist interpretation of the more rapid rate of molecular evolution in a living fossil is possible' because we may expect more rapid molecular evolution 'accompanying slow morphological changes because the reduced number of loci undergoing substitutions may sustain more intense selection per locus'.

Perhaps this is so, but selectionists can cite scripture for their own purpose. Neutralists, however, are delighted to find that this molecule of a living fossil underwent changes at least as rapidly as the

homologous molecules in highly-evolved species. This supports the thesis that 'there seems to be considerable latitude at the molecular level for random genetic changes that have no effect on the fitness of the organism' (ref. 2).

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¹ Stenzel, P., *Nature*, **252**, 62 (1974).

² King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).

Sunspot cycle periodicities

THE Maximum Entropy Spectral Analysis technique of Burg (unpublished) and Cohen and Lintz¹ has led to the discovery of periodicities in sunspot data and an ability to predict ionospheric reflectivity and climatic features. But the theory that a planetary influence affects sunspot variations^{2–4} still cannot be ruled out, as is shown by a further analysis of the data of ref. 1.

I accept the 11.05 ± 1.5 , 9.8 ± 0.1 , and 8.15 ± 0.15 yr terms found by Cohen and Lintz but wish to choose another way of describing the cause of their 89.6 yr peak. The proposed fundamental beat tone of 179 yr just does not appear in their periodogram nor is it the beat period suggested. At about one half that value (also the true value of the beat period they have proposed) there is a fairly broad resonance. But 89.6 yr (really more like 86 skewed) is the value one obtains when oppositions and conjunctions of Uranus and Neptune are considered as a possible cause. The relative phase of these two planets is at quadrature during the peak sunspot maxima of 1778, 1864 and 1950 and in conjunction or opposition at the least sunspot maxima of 1821, 1907 and 1993 (predicted). I found the same periodicity but different significant phase relationships when I compared the relative positions of Uranus and Neptune to peak periods of energy released by terrestrial earthquakes². Because of the special phase relationships between the sunspot and planetary data, I feel the 86 yr 'tone' is really due to Uranus and Neptune.

Interestingly, the other periodic terms which Cohen and Lintz found can be explained as the resonance of the following planetary mean motions: (1) $J + S + U - N = 1/8.06$ yr; (2) $J + U + N = 1/9.78$ yr; (3) $J + U - N = 1/11.09$ yr.

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¹ Cohen, T. J., and Lintz, P. R., *Nature*, **250**, 398–399 (1974).

² Bagby, J. P., *The Moon*, **6**, 398–404 (1973).

³ Bigg, E. K., *Astr. J.*, **72**, 463 (1967).

⁴ Wood, K. D., *Nature*, **240**, 91–93 (1972).

Matters arising

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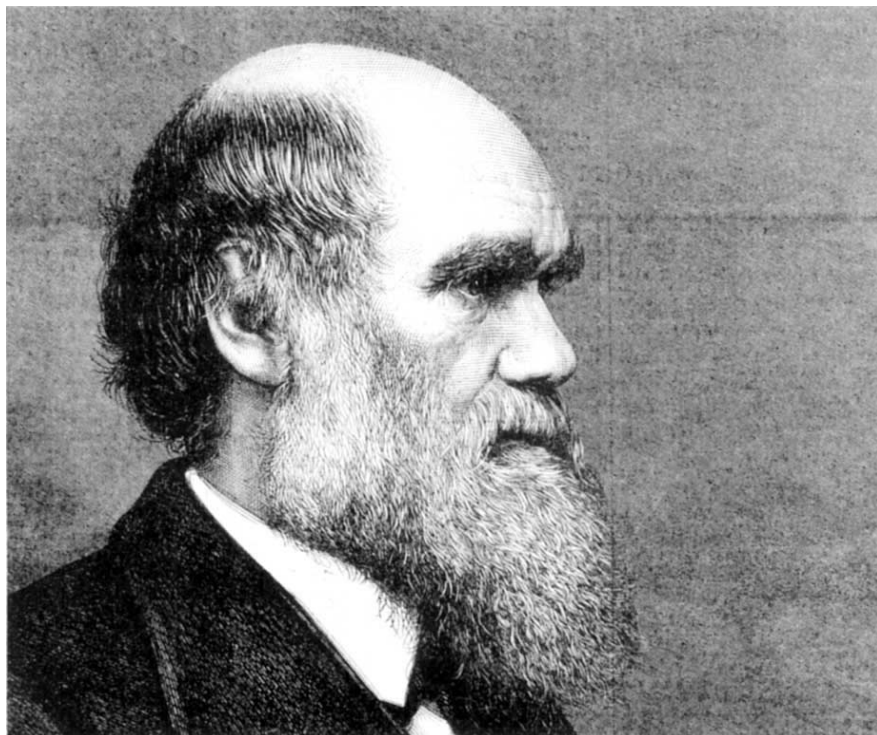
reviews

Missing some of Darwin's brilliance

Darwin on Man: A Psychological Study of Scientific Creativity. By Howard E. Gruber. (Together with Darwin's early and unpublished notebooks, transcribed and annotated by Paul H. Barrett.) Pp. xxv+495. (Wildwood House: London, September 1974.) £5.00.

HOWARD E. GRUBER and Paul H. Barrett have produced a book which they hope will elucidate the roots of Charles Darwin's achievement. They believe that the six notebooks which Darwin kept in the years 1837-39 contain crucial evidence for an analysis of his scientific creativity. Sir Gavin de Beer and his collaborators have already published the four notebooks on the transmutation of species. The Gruber-Barrett volume now presents for the first time a complete transcription of Darwin's two notebooks (designated M and N) on man, mind, and materialism, along with several of his other unpublished contemporary documents on the same subjects. They also reprint some crucial passages from previously published documents, primarily the notebooks on transmutation of species. Barrett transcribed the manuscript materials and has provided detailed bibliographic notes, which are supplemented by Gruber's useful commentaries summarising both the M and N notebooks.

Gruber's primary contribution to the volume is a 257 page analysis of Darwin's scientific creativity, in which the M and N notebooks play a prominent role. Gruber presents two major theses. He first argues that Darwin's invention of the theory of evolution by natural selection between 1837 and 1839 was not the result of a "golden moment of insight", but was instead the culmination of a gradual process involving many levels of thought. This illustrates the belief of Gruber (and Jean Piaget, who wrote a laudatory foreword to the book) that creativity is generally a more gradual process than most people realise. His second thesis is that the M and N notebooks hold "the key to understanding the essential role of his ideas about man and mind in his thinking about evolution". Gruber concludes



Charles Darwin: spent his life refining the original theory

that Darwin's materialism is evident in these notebooks, and that he delayed publication of his conception of evolution by natural selection because he feared persecution and ridicule for his materialism.

Gruber's thesis that Darwin's creation of the theory of evolution by natural selection was a gradual and cumulative process is convincing. But scientific creativity is elusive, and the details of Gruber's analysis are fraught with difficulties. His chapters on the Darwin family *Weltanschauung* and Darwin's teachers contain precious little evidence about actual influences upon Darwin himself. The crucial evidence about his scientific creativity comes from the notebooks. Yet what are these notebooks? They contain Darwin's hurriedly written impressionistic speculations, including his gleanings from other literature mixed indistinguishably with his own ideas. Many of the passages, especially in the M and N notebooks, are unclear or contradictory. We have no way of knowing which passages Darwin considered important or trivial at the time that he wrote them. We can, however, agree with Darwin's later assessment that much in the notebooks is trivial or worthless, even for the historian. To

recreate Darwin's creativity from these notebooks is terribly difficult.

To simplify matters, Gruber explicitly ignores Darwin's ideas about heredity, a serious omission which he acknowledges. He then proceeds, like a classical scholar working on Anaximander's one fragment, to make much of little. He finds in Darwin's notebooks an "underlying order" which completely escapes me. In constructing logical systems out of Darwin's speculations, he is led into contradictions. For example, Gruber argues that Darwin's brief mention of "monads" is really a whole theory of evolution, and that the "monad theory contains the principle of spontaneous generation". Later, Gruber argues that Darwin gave up the theory of spontaneous generation, influenced by Ehrenberg's claim that extant lower organisms were identical with fossil forms. The problem with this scenario is that Ehrenberg is mentioned in the very first passage of Darwin's supposed 'monad theory'. So although Gruber's thesis about the gradual nature of Darwin's creativity is reasonable, the details of his creative process remain obscure.

Gruber's second thesis, that the M and N notebooks and associated docu-

ments contain much about Darwin's views on man, mind, and materialism in relation to evolution, is certainly true. Indeed, the major significance of this book lies in showing that at a very early stage Darwin understood that his idea of evolution by natural selection had vast materialist implications for human evolution and psychology.

Yet Gruber's conclusion that Darwin put off publication of his ideas primarily from fear of persecution and ridicule for his materialist ideas is drawn from shaky evidence. Gruber even resorts to interpreting a dream briefly reported by Darwin, and argues that a photograph of Darwin taken in 1854 reveals "the strain of long years of delay".

A different interpretation is possible. Darwin had high standards for his published work. He later commented that he benefitted from the delay in publishing his works on evolution because he was able to gather better evidence and think through possible objections. Perhaps he was motivated not so much by fear of ridicule as a materialist, as by the feeling that his own standards of argument and evidence had not been attained.

After giving the impression that he admires Darwin's materialism, Gruber's last chapter, "Creative Thought: The Work of Purposeful Beings", contains a surprise. His theory of creativity is vitalistic nonsense. It follows the tradition of Alfred Russel Wallace's mysticism (after 1864) rather than Darwin's materialism. Gruber's theory is that each individual organism functions "according to its own internal laws or organisation". When two organisms interact, the result is

behaviour which is utterly unpredictable from a consideration of the special laws governing the individual organisms. A human has, in addition, an internal unpredictable creativity resulting from the interaction of the separate parts of his thought, each of which has a life of its own. So in the end the reader discovers that in his analysis of Darwin's creativity, Gruber is conducting the inquiry under the assumption that the creative process is inherently inscrutable. Darwin spent his life reducing complex biological behaviour to materialist laws. He intensely disliked Wallace's mysticism about human minds, and he would have been appalled to have Gruber's vitalistic theory applied to himself.

Does this book as a whole open up a fruitful field of Darwin research? I hope not. By focusing almost entirely upon Darwin's theory construction of 1837-39, the authors have missed the most important and influential part of Darwin's genius. As he himself believed, Darwin is important not because he outlined the theory of evolution by natural selection in the late 1830s, but because for the rest of his life he refined and supplemented the theory, found evidence for it, and presented it in enormously influential published works. Michael Ghiselin's *The Triumph of the Darwinian Method* (The University of California Press: Berkeley and London, 1973) is a step in the right direction, but the vast and important problem of Darwin's influence through his letters and published works in the second half of the nineteenth century has scarcely been touched. The M and N notebooks seem inconsequential in comparison.

William B. Provine

Elementary world climatology

World Climatology. By John G. Lockwood. Pp. xiv + 330. (Arnold: London, March 1974.) £8.50.

THE study of climatic change and its effect on the environment is one of the most important branches of modern science, in terms of the potential direct effects on the lives of non-scientists. As a result of developments in several fields including studies of the Earth's magnetic field and astronomy, as well as the more conventional aspects of meteorology (which now include use of satellite observations and high speed computers), a synthesis leading to an understanding of at least some aspects of climatic change seems to be in the offing. As a result, many people who have no formal training in meteorology are working in areas where some detailed understanding of the working of the atmosphere is essential. Those people would find this book invaluable.

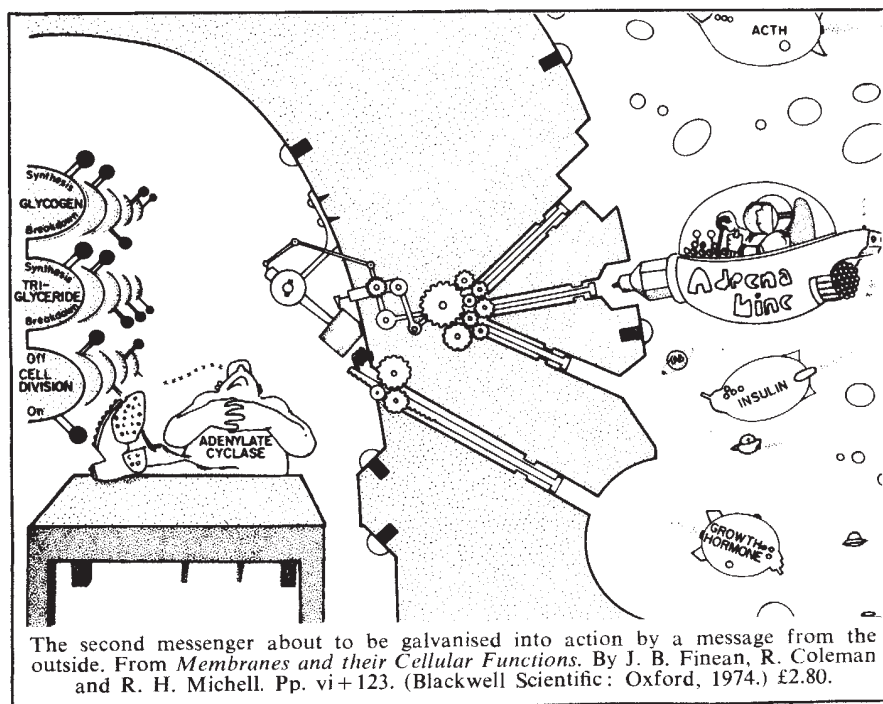
World Climatology is not concerned with climatic change at all, but with the physical basis of climatology with particular reference to environmentally important aspects. It is intended, says the author "for students taking university courses on climatology, particularly those at an advanced level" and the reader is warned that an acquaintance with basic concepts of meteorology, mathematics and physics is required. But these assumed basics are, in fact, at the most elementary level, and the book introduces gently such concepts as the Stefan-Boltzmann law of black body radiation.

The first part of the book deals with the basics of climatology: radiation laws, convection and turbulence, precipitation and evaporation, and local microclimates (ranging down to that at the surface of a plant leaf). The second and third parts apply these basics to the low latitude atmosphere and to the atmosphere of middle and high latitude regions. With a generous sprinkling of figures—one or more clear and informative line drawings on most pages—and well written text this makes the book ideally suited for its intended readership, but equally valuable to anyone trying to fill in a knowledge of climatology which is sketchier than it should be.

The publishers of this book have done an excellent job. The clarity of the figures is matched by the clarity of the text. There is a generous bibliography, in the form of references at the end of each chapter.

This book is a good place to find out about the basics of climatology.

John Gribbin



The second messenger about to be galvanised into action by a message from the outside. From *Membranes and their Cellular Functions*. By J. B. Finean, R. Coleman and R. H. Michell. Pp. vi + 123. (Blackwell Scientific: Oxford, 1974.) £2.80.

The Physics of Time Asymmetry. By P. C. W. Davies. Pp. xviii+214. (Surrey University Press; Leighton Buzzard, 1974.) £6.50.

THIS book is about what is popularly called the 'arrow of time' but the author does not much like the term because the asymmetry is that of the world in respect to time, not of time itself. The book is a critical study of the origin of the asymmetry and of its role in basic physics. Indeed, it could serve as the making of an excellent course on the foundations of physics.

With the only known exception of properties of K mesons, the laws of physics are symmetric in regard to time, and so the asymmetry must arise from boundary conditions. Davies proceeds from that consideration to two important inferences. First, that irreversibility in the Universe depends ultimately on gravitation, since any sizeable body in the Universe is held together by its own gravitation and there is no permanent equilibrium state for such a body. Second, that all important aspects of time asymmetry are traceable to the beginning or end of the Universe. Both bring the subject naturally into the realm of cosmology. Appropriately, the chapter in the centre of the book is on "Thermodynamics and cosmology". Moreover, the fact that in ordinary electromagnetism we consider only retarded effects is nowadays generally explained by the 'absorber' theory of radiation, and so it again relates the subject to cosmology. Whether or not the thermodynamic and electromagnetic properties are independent seems to remain an open question. Davies discusses all that in his next chapter. Thermodynamic irreversibility is bound up with probability theory, and a more fundamental sort of probability is involved in quantum mechanics, as is then described. Finally, Davies discusses the bearing of the whole subject upon the choice of a cosmological model.

Davies skilfully conveys the flavour of the mathematical treatment without going into its details. His presentation tends to be over-modest, and this inhibits him somewhat from stating categorically the conclusions reached at each stage of the development of the topic—the reader has to do a little searching to find them. Nevertheless, "Having completed this book", he makes bold to conclude that (Introduction, page 7), "the basic framework for understanding the nature of time asymmetry is clearly available, and it is unlikely that any future considerations of detail will introduce anything qualitatively new". Although the book certainly seems to do more than any

other towards achieving this happy outcome, one is left with an uneasy feeling that something is still missing. The old problem of the relationship between the indeterministic world of quantum physics and the fully deterministic world of general relativity seems still imperfectly understood. Actually, Davies exposes the difficulties in his section 6.3, "Quantum measurement theory". I cannot but conjecture that the place of the observer will somehow have to be recognised more extensively in formulating physical theory and that this will entail the admission of uncertainties associated with other physical constants besides Planck's constant.

The book most adroitly brings together all the main relevant lines of thought; whatever remains to be done must surely start from the ground so well surveyed by Dr Davies.

W. H. McCrea

Time and space

Space, Time and Spacetime. By Lawrence Sklar. Pp. xii+423. (University of California Press: Berkeley, Los Angeles and London, 1974.) \$15.00; £7.50.

LAWRENCE Sklar is a philosopher writing about philosophical aspects of space and time, from Newton and Leibniz to Einstein and Reichenbach. Nevertheless, his clear grasp of modern mathematics and physics, and his lucid exposition, make this book eminently suitable for physicists and mathematicians who wish to explore the philosophical background to their subject. Although the text is not in any sense mathematical, crucial topological and geometrical concepts are carefully explained before they are incorporated into the author's thesis.

The book is divided into four main sections. The first deals with the epistemology of geometry with a historical development leading up to Minkowskian and Riemannian spacetimes. Much attention is then devoted to Poincaré's proposition that the geometrical structure of the world is merely a matter of convention and to the existence of different hypotheses each equally capable of explaining the same observations. The complex web of argument and counter argument subdivides into many alternative points of view and may well frustrate the scientific reader who probably regards the whole issue as sterile overemphasis, especially as the author terminates the controversy with an admission of inconclusiveness.

Part two is concerned with absolute motion and the theory of relativity, tracing the controversy between the substantialist view of Newton that space is an entity and the relational views of Leibniz and Mach that it's not there at all. The special and general theories of relativity are somewhat grafted on to this discussion without, of course, solving the problem. Sklar gives a fine descriptive account of the theories of relativity, which could well be used by physics students wishing simply to learn the subject. Even modern approaches such as geometrodynamics get a mention. At the end of this section the author presents his own rather unconvincing answer to the problem of the origin of inertial forces. Rejecting Mach for apparently aesthetic reasons and wishing to retain a pure relationist stance, he proposes that the statement "is absolutely accelerated" is not a relational term, giving it instead the status of a complete assertion. This may well be a valid philosophical contortion, but it is unlikely that the physicist will be satisfied with the denial of any explanation for inertial forces.

Part three of the book deals mainly with time—the usual problems of simultaneity, time dilation, twins paradox and so on in relativity theory—and also presents some bold treatment of bizarre topologies, including a discussion of trouser spaces, non-orientable manifolds and closed time-like world lines. The discussion is framed in the causal theory of time to which the last part of this section turns, concluding with the widely held belief that the theory is implausible.

The final section summarises briefly the fascinating and controversial subject of time asymmetry. The account suffers somewhat from superficiality, and the vital topic of Reichenbach's branch systems is not discussed in detail. The conclusion given to this section is that we simply 'know' the relation of temporal priority between events. This is a most unsatisfactory escape route, which does nothing to dispel the lamentable confusion which exists between the phenomenon of the psychological flow of time and time asymmetry as encountered in the objective world, and which is so prevalent among scientists and philosophers who write about time asymmetry.

This book is a comprehensive, well written and authoritative account of space and time which well suits both scientists and philosophers of science. It is inclined to be a little tedious and repetitive, but contains plenty of exciting and interesting material for students of these disciplines. P. C. W. Davies

obituary

Herbert Alexander Sober, a major innovator and developer of our current technology for the separation and purification of biologically important macromolecules, died of a heart attack in Washington, D.C., on November 26, 1974, following a relatively minor operation.

Sober was an undergraduate at the City College of the City of New York and received his Ph.D. degree in 1942, majoring in biochemistry at the University of Wisconsin. Following his graduate studies, he spent three years as a toxicologist and as Chief of the Analytical Research Group at Edgewood Arsenal near Baltimore. After two years at Mount Sinai Hospital in New York he joined the National Cancer Institute, NIH, as a member of the Commissioned Corps of the US Public Health Service, serving as Chief of the Laboratory of Biochemistry. In 1968 he transferred to the US Civil Service and took the position of Chief in the Laboratory of

Nutrition and Endocrinology, National Institutes of Arthritis, Metabolism, and Digestive Diseases. Sober is best known as co-developer, with Elbert Peterson, of the modified cellulose ion exchangers. These substances, which could be prepared in a wide variety of forms having either cationic or anionic characteristics, and possessing a wide range of intrinsic pK values, are extremely gentle to sensitive protein and nucleic acid systems and also exhibit excellent discrimination when combined with the principle of gradient elution for the separation of macromolecular mixtures. There are very few papers published in the biochemical or molecular biological literature that do not quote the use of the cellulose derivations as key tools in the experiments described. The value of these chromatographic techniques was recognised, in 1970, by the selection of Sober and Peterson for the Hillebrand Award, presented by the American Chemical Society of Washington.

The association of Sober's name with chromatography and its applications has tended to obscure his contributions to a number of other areas. He published nearly 150 papers during his career, including the ever-useful *Handbook of Biochemistry*. He was, for example, involved in the first isolation of pure crystalline pyridoxamine and pyridoxal phosphates. In the last few years he and his colleagues, particularly Dr Robert Simpson and G. W. Rushizky, made major advances in our understanding of the interaction between basic oligopeptides and polynucleotides, particularly when applied to research on the chemical nature of chromatin.

Herbert Sober was, above all, a man of great balance and human warmth. He was universally respected by his coworkers and students over the years and maintained, throughout his professional life, a blend of vigorous interest in science and a rare ability to share his love, wisdom, and simplicity with family and friends alike.

announcements

Appointment

Alasdair D. Berrie, Officer-in-charge of the River Laboratory of the Freshwater Biological Association at East Stoke, Wareham, Dorset, has been accorded the title of Visiting Professor in the University of Reading.

Miscellaneous

Biogeography Study Group. At the annual conference of the Institute of British Geographers, this Group was formally constituted. It aims to foster the study and development of biogeography by discussion and field meetings, and by providing contact with those people working in related disciplines. A symposium is scheduled for September, 1975, to be held on the North York Moors. Further information may be obtained from: Dr L. F. Curtis, Department of Geography, University of Bristol, Bristol, UK; Mr J. A. Taylor, Department of Geography, University College of Wales, Aberystwyth Dyfed SY23 3DB, Wales; Secretary, Institute of British Geographers, 1 Kensington Gore, London SW7 2AR, UK.

Fractionation Services. The American Red Cross National Fractionation Center, supported by the National Heart and Lung Institute, is offering facilities to an investigator, for the pilot-plant or large-scale production of blood proteins, enzymes, membrane fractions and so on. Further information from: Dr M. Wickerhauser, Director, American Red Cross National Fractionation Center, 9312 Old Georgetown Road, Bethesda, Maryland 20014.

METASERV prize. The Institute of Metallurgists has organised a competition in Metallurgy with prize money totalling £200 (including a special student Prize). The closing date for the competition is July 31, 1975. Further details may be obtained from: *The Metallurgist* or The Institution of Metallurgists, Northway House, High Road, Whetstone, London N20 9LW, UK.

International meeting

April 27-May 3, **Stereochemistry**, Lucerne, Switzerland (Professor J. Dale, Department of Chemistry, University of Oslo, Blindern, Oslo, Norway).

Reports and publications

Great Britain

UMIST 1824-1974. Pp. 104. (Advance No. 15.) (Manchester: University of Manchester Institute of Science and Technology, 1974.) [512]
Scholarships Guide for Commonwealth Postgraduate Students, 1975/1977. Pp. 310. (London: The Association of Commonwealth Universities, 36 Gordon Square, WC1, 1974.) £2.50. [612]
The Zoological Record, 1970, Vol. 107, Section 1: Comprehensive Zoology. Compiled by J. Fitzgibbon. Pp. vi + 68. £3.65. 1970, Volume 107, Section 3: Porifera together with Archeocyatha. Compiled by S. Ware. Pp. v + 16. £3.65. 1970, Volume 107, Section 5: Echinodermata. Compiled by A. M. Clark. Pp. v + 51. £5.15. (London: The Zoological Society of London, 1974.) [612]
Agricultural Research Council; and Medical Research Council. Food and Nutrition Research: Report of the ARC/MRC Committee. Pp. xv + 211. (London: HMSO; Amsterdam and New York: Elsevier Scientific Publishing Company, 1974.) £3.80 net. [912]
The Responsibility of the Governors. By Sir Michael Swann. FRS. (BBC Lunch-Time Lectures, Ninth Series, 1.) Pp. 14. (London: BBC, 1974.) [912]
A Second Appendix to the Second Edition of an Index of Mineral Species and Varieties Arranged Chemically. By Max H. Hey and Peter G. Embrey. Pp. xii + 168. (London: British Museum, (Natural History), 1974.) [912]

Other countries

Bibliography of Laser Doppler Anemometry Literature. Edited by F. Durst and M. Zare. Pp. 55. (Copenhagen: DISA Elektronik; Bristol, DISA UK, Techno House, Redcliffe Way, 1974.) gratis. [1911]
Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 23: Laboratory Procedures for Cation Exchange Measurements in Soils. By B. M. Tucker. Pp. 46. (Melbourne: CSIRO, 1974.) [1911]
National Research Council Canada. NRCC No. 13686: A Method of Monitoring Nationally for Possible Delayed Effects of Various Occupational Environments. By H. B. Newcombe. (Associate Committee on Scientific Criteria for Environmental Quality.) Pp. 42. (Ottawa: National Research Council, 1974.) [1911]